The Impact of Hypoxia on Aerobic Exercise Capacity

Dissertation

zur
Erlangung der naturwissenschafltichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der
Mathematisch-naturwissenschaftlichen Fakultät
der
Universität Zürich

von
Christoph Andreas Siebenmann
aus
Aarau AG

Promotionskomitee
Prof. Dr. Carsten Lundby (Vorsitz)
Prof. Dr. Max Gassmann
Dr. Paul Robach

Zürich, 2012

Contents

Summary III

Zusammenfassung V

Acknowledgments VII

1. Introduction 1

1.1. Background 1

1.2. Acute Hypoxia and aerobic exercise 2

Direct implication of a reduced PO_2 for aerobic exercise 2

Aim 1: Limitations induced by the pulmonary circulation 5

Aim 2: Limitations induced by the cerebral circulation 7

1.3. Chronic Hypoxia and aerobic exercise 10

Aim 3: The effect of Live High – Train Low on elite athletes 11

2. Manuscripts 13

2.1. Dexamethasone Improves Maximal Exercise Capacity of Individuals Susceptible to High Altitude Pulmonary Edema at 4559m 14

2.2. Maximal exercise capacity in individuals susceptible to high altitude pulmonary edema at 4559 m 23

2.3. Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O_2 uptake 35

2.4. "Live high–train low" using normobaric hypoxia: a double-blinded, placebo-controlled study 53

2.5. The role of haemoglobin mass on VO_2max following normobaric 'live high-train low' in endurance-trained athletes 65

3. Discussion and outlook 72

3.1. Limitations to exercise in acute hypoxia induced by the pulmonary circulation 72

3.2. Limitations to exercise in acute hypoxia induced by the cerebral circulation 73

3.3. The effect of Live High – Train Low on exercise capacity of elite athletes 73

4. Bibliography 75

5. Curriculum Vitae 84

Summary

Acute hypoxia impairs aerobic exercise by reducing the capacity for maximal O_2 uptake (VO_2max). This is mainly the consequence of a lower arterial O_2 content (c_aO_2) and, in severe hypoxia, cardiac output during maximal exercise as the combination of these two factors attenuates convective O_2 supply to the exercising muscle cells. Nevertheless, the incapacitating effect of hypoxia is partially restored during prolonged exposure as an increased renal erythropoietin release induces polycythemia that normalizes c_aO_2. Since this mechanism may benefit performance not only in hypoxia but also in normoxia different forms of altitude training were developed all aiming to enhance athletic performance at sea level.

The purpose of the present project was to enhance our understanding regarding the interaction between both, acute and chronic hypoxia and aerobic exercise. In acute hypoxia the contribution of the intrinsic responses of the pulmonary and the cerebral circulation to the reduction in VO_2max was investigated. Specifically, we hypothesized that the hypoxia-induced rise in pulmonary artery pressure induces exercise limitations by increasing right ventricular afterload and/ or promoting pulmonary ventilation-perfusion mismatch. Furthermore, we suggested that the cerebral hypoxia that develops during exercise at altitude would limit VO_2max by accelerating the development of supraspinal fatigue. Regarding chronic hypoxia we tested the efficacy and underlying mechanisms of the contemporary altitude training strategy, i.e. the Live High – Train Low approach, on elite endurance athletes in a double-blinded and placebo-controlled study design.

The results collected in three independent studies revealed the following:

1) At 4,559 m altitude pulmonary vasodilation induced by the glucocorticoid Dexamethasone elevates VO_2max of individuals present with an excessive vasoconstrictive response to hypoxia without affecting arterial O_2 saturation (S_aO_2). A direct comparison to normal individuals further suggested a larger hypoxia-induced exercise impairment in these individuals but also no differences in S_aO_2 during maximal exercise. We thus conclude that hypoxic pulmonary vasoconstriction may contribute to the reduced VO_2max in acute hypoxia potentially by increasing right ventricular afterload and thereby attenuating cardiac output.
2) Although administration of CO_2 during exercise at 3,454 m altitude elevated cerebral blood flow and, as a consequence, cerebral oxygenation, VO_2max remained unaffected. This indicates that the contribution of the reduced cerebral oxygenation on the limitation

of VO_2max in hypoxia is neglectable. Nevertheless, as a VO_2max test induces progressive demand for muscular O_2 supply, the capacity of the O_2 transport system might have been exhausted before supraspinal fatigue occurred, and thus we cannot exclude that the decline in cerebral oxygenation may play a role during submaximal exercise in hypoxia.

3) Four weeks of discontinuous (16 hours per day) exposure to normobaric hypoxia corresponding to 3,000 m combined with daily training in normoxia failed to benefit the performance of elite endurance athletes. This was explained by the absence of an effect of hypoxia on total red cell volume. These findings suggest a potential role of a placebo-effect in earlier studies and indicate that four weeks of discontinuous hypoxic exposure may be insufficient to induce physiological adaptations. Athletes should take this into consideration before shouldering the inconveniences associated with Live High – Train Low altitude training.

In brief, the present results support a limiting role of pulmonary vasoconstriction but not of attenuated cerebral oxygenation on VO_2max in hypoxia. They further indicate that altitude training following the Live High – Train Low strategy may not be superior to conventional endurance training.

Zusammenfassung

Akute Hypoxie führt zu einer Reduktion der maximalen O_2 Aufnahmekapazität (VO_2max) und beeinträchtigt dadurch die aerobe Leistungsfähigkeit. Die Hauptursache für die Abnahme von VO_2max ist ein verringerter O_2 Transport zu der arbeitenden Muskulatur, der durch einen tieferen arteriellen O_2-Gehalt (c_aO_2) und in schwerer Hypoxie zusätzlich durch ein vermindertes maximales Herz-Minuten-Volumen zustande kommt.
Bei chronischer Hypoxie-Exposition führt jedoch eine verstärkte renale Erythropoietin-Ausschüttung zu einer Erhöhung der Hämoglobinkonzentraion im Blut. Dadurch normalisiert sich c_aO_2 allmählich, wodurch sich auch die aerobe Leistungsfähigkeit teilweise erholt. Da eine solche Anpassung des Blutes allerdings nicht nur die Leistungsfähigkeit in Hypoxie, sondern auch die in Normoxie verbessern könnte, erhoffen sich viele Athleten einen Wettkampfvorteil aus dem Akklimatisationsprozess. Dazu wurden verschiedene Formen von Höhentraining entwickelt, die alle eine Verbesserung der Leistungsfähigkeit in Normoxie anstreben.

Das Ziel dieses Projekts war es, die Auswirkungen von akuter und chronischer Hypoxie auf die aerobe Leistungsfähigkeit zu untersuchen. In akuter Hypoxie wurde getestet, ob die spezifischen Reaktionen von Lungen- und Hirnkreislauf zu der Verminderung von VO_2max beitragen. Wir vermuteten, dass die pulmonale Vasokonstriktion in Hypoxie VO_2max beinträchtigt, indem sie die rechtsventrikuläre Nachlast erhöht und ein optimiertes Ventilations/ Perfusions-Verhältnis verhindert. Weiterhin spekulierten wir, dass die verminderte O_2 Versorgung des Gehirns die Entstehung von supraspinaler Ermüdung beschleunigt und dadurch eine raschere Erschöpfung erzwingt.
Hinsichtlich chronischer Hypoxie untersuchten wir die Wirkung der vielverspechendsten Höhentrainingsstrategie (Hoch leben – Tief trainieren) auf die Leistungsfähigkeit von hochtrainierten Ausdauerathleten, sowie die Mechanismen, die einer eventuellen Verbesserung unterliegen.

Die folgenen Ergebnisse stammen aus drei unabhängigen Studien:

1) Bei Probanden, die in akuter Hypoxie zu exzessiver pulmonaler Vasokonstriktion neigen, verbesserte eine durch das Glukokortikoid Dexamethason bewirkte pulmonale Vasodilatation VO_2max auf einer Höhe von 4‘559 m.ü.M. Dies geschah ohne eine Erhöhung der arteriellen O_2-Sättigung (SaO_2) bei maximaler Belastung. Weiterhin tendierten diese Probanden im direkten Vergleich zu einer Kontrollgruppe zu einer

stärkeren Verringerung von VO_2max in Hypoxie, wobei auch hier keine Unterschiede in SaO_2 bei maximaler Belastung vorlagen. Aus diesen Ergebnissen schliessen wir, dass die pulmonale Vasokonstriktion in akuter Hypoxie zur Verringerung von VO_2max beiträgt. Die Ursache könnte eine gesteigerte rechtsventrikuläre Nachlast sein, die das Herz-Minuten-Volumen reduziert.

2) Auf 3‘454 m.ü.M. erhöhte eine inspiratorische Administration von CO_2 die arterielle Blutversorgung und damit die Oxygenierung des Gerhirns. Entgegen unserer Hypothese hatte diese jedoch keinen Effekt auf VO_2max. Dies zeigt, dass die Abnahme der cerebralen Oxygenierung bei der Verringerung von VO_2max in Hypoxie keine entscheidende Rolle spielt. Allerdings könnte die zunehmende Belastung während der VO_2max Tests und der damit verbundene ansteigende O_2 Bedarf in den Skelettmuskeln die Kapazität des O_2 Transportsystems überfordert haben, bevor bevor die cerebrale Hypoxie zu supraspinaler Ermüdung führte. Daher kann nicht ausgeschlossen werden, dass die verminderte cerebrale Oxygenierung in Hypoxie die Leistungsfähigkeit in submaximalen Tests beeinflussen könnte.
3) Vier Wochen diskontinuierliche normobare Hypoxie-Exposition (16 Stunden/ Tag, entsprechend 3‘000 m.ü.M.) bewirkten keine Erhöhung des Gesamtvolumens der Erythrozyten von hochtrainierten Ausdauerathleten und führte daher zu keiner Leistungsverbesserung. Dies lässt vermuten, dass Athleten in früheren Studien von einem Placebo-Effekt profitiert hatten. Weiterhin zeigt es, dass vier Wochen diskontinuierliche Hypoxie möglicherweise nicht genügen um eine physiologische Adaptation zu erwirken. Athleten sollten sich dessen bewusst sein, bevor sie sich entscheiden die Unannehmlichkeiten eines Höhentrainings auf sich zu nehmen.

Insgesamt zeigen die vorliegenden Resultate, dass die pulmonale Vasokonstriktion, nicht aber die verminderte cerebrale Oxygenierung VO_2max in Hypoxie beeinträchtigen. Weiterhin deuten sie darauf hin, dass Höhentraining nach der „Hoch leben – Tief trainieren“ Strategie keine grössere Leistungssteigerung zur Folge hat als konventionelles Ausdauertraining.

Acknowledgments

I would like to thank the following persons:

Prof. Dr. Carsten Lundby for supervising my PhD-project and for sharing his exceptional knowledge in this field.

Prof. Dr. Max Gassmann for attendance in my PhD-committee and for all his help and advice.

Dr. Paul Robach for attendance in my PhD-committee and for his practical support particularly during the Live High – Train Low study.

Dr. Peter Rasmussen for many suggestions that helped to improve my work and for statistical advice.

Robert Jacobs for his friendship and humour that lightened many long days in the office.

Maja Schlittler for her love and her never-ending support, patience and understanding.

My family for all the encouragement and support that helped me to get to this point.

Finally, all the persons that volunteered as subjects in the studies of my PhD-project.

1. Introduction

1.1 Background

The reduced O_2 availability in hypoxia has a variety of effects on the human organism whereof one of the most noticeable is an impairment of aerobic exercise capacity. While the extent of this is largest in the initial phase of the hypoxic exposure, it attenuates with time as different physiological adaptive mechanisms partially restore uptake and transport of O_2 to the skeletal muscles.

The factors underlying the impeding impact of hypoxia have traditionally been of great interest in medicine and physiology as these do not only affect healthy individuals at altitude, but also a large number of patients suffering from conditions that abate internal O_2 availability (55). However, despite a long history of research (25, 27, 134) our knowledge in this field remains far from complete.

The purpose of the present PhD-project was to gain new insights into the interaction between hypoxia and aerobic exercise. To better understand the limitations induced by acute hypoxia, the contributions of two mechanisms other than those conventionally assumed responsible were investigated. Regarding the effect chronic hypoxia, the process of acclimatization and the resulting implications for subsequent sea level performance were studied in endurance athletes conducting the contemporary form of altitude training.
After a brief review of the present state of knowledge in each respective field, the results are presented and discussed in five manuscripts (102, 114-117), four of which are to date in press or published in peer-reviewed journals.

1.2 Acute hypoxia and aerobic exercise

The incapacitating effect of hypoxia has been recognized ever since humans started to ascend to altitude, but for a long time the underlying mechanisms were not understood. In the beginning of the 20th century systematic investigations identified the reduced partial pressure of O_2 (PO_2) rather than the barometric pressure per se as the perpetrator (35, 143), and subsequently a long list of studies have revealed how this may impair aerobic exercise capacity.

Direct implication of a reduced PO_2 for aerobic exercise

Performance in tasks that rely on aerobic metabolism is related to three components: i) maximal oxygen uptake (VO_2max), which sets the notional upper limit for aerobic performance, ii) the fraction of VO_2max that is sustainable for the required time and yields the "performance VO_2" and iii) exercise efficiency, which determines the work rate that is generated at the O_2 cost corresponding to the performance VO_2 (figure 1) (17, 65). While the latter two components are not affected by acute hypoxia (42, 140) VO_2max decreases with the inspired PO_2 in a curvi-linear relationship (figure 2) (30, 34, 44, 92). To understand how acute hypoxia impairs aerobic exercise it is thus necessary to distinguish the factors determining VO_2max and how they are affected by the reduction in PO_2.

During incremental whole body exercise pulmonary VO_2 rises linearly due to an accelerating aerobic metabolism in the active skeletal muscles (59). However, as VO_2max is reached near maximal effort, the VO_2 curve plateaus and becomes irresponsive to further elevations in workload (13, 59). This indicates failure to either increase muscular O_2 supply or O_2 consumption by the mitochondria. If an exercise task involves a large muscle mass, as it is required for the achievement of whole body VO_2max, mitochondrial capacity clearly exceeds the highest rate of O_2 delivery (20) and thus VO_2max is limited by O_2 transport rather than utilization.

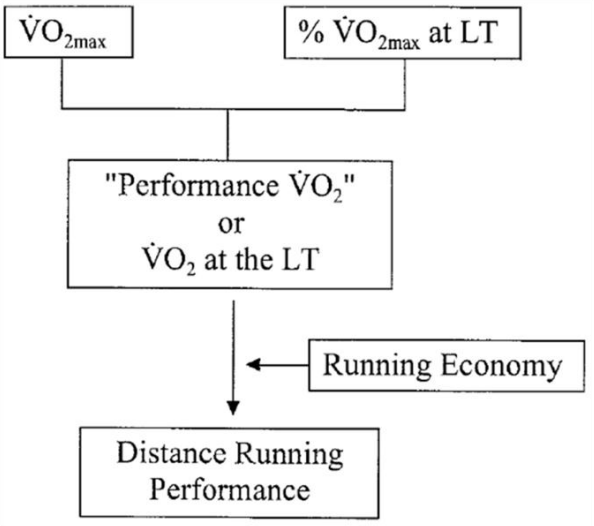

Figure 1: Linkage between VO_2max, the percent of VO_2max that can be sustained for a prolonged time (i.e. VO_2 at the lactate threshold (LT)) and running economy as they relate to distance running performance. Reproduced from Bassett et al., 2000.

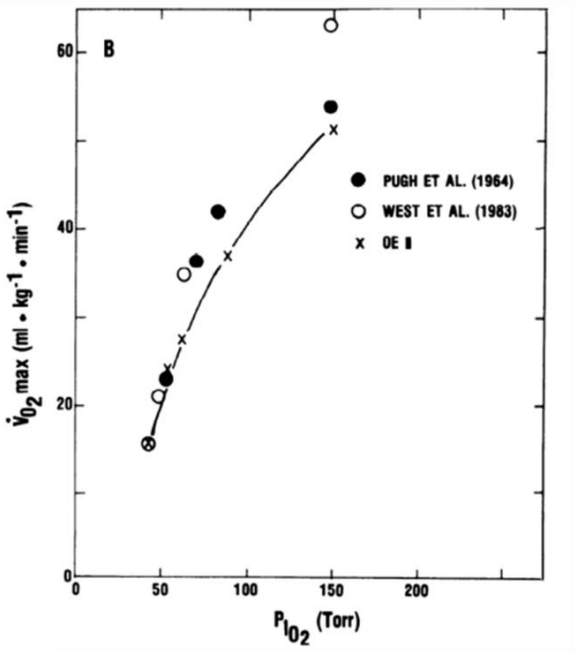

Figure 2: Relationship between VO_2max and the inspired PO_2. Reproduced from Cymerman et al. 1989.

To reach the muscle's mitochondria O_2 is carried along a cascade of four consecutive processes, i.e. pulmonary ventilation, alveolar-capillary diffusion, convective transport by the circulation and capillary-mitochondrial diffusion (27, 133). While pulmonary ventilation and convective O_2 transport are active processes that are stimulated as the demand for O_2 increases, diffusive transport at the pulmonary and the muscular sites is passively driven by the PO_2 gradient over the according membranes. These steps are challenged during exercise as the increasing cardiac output shortens capillary transit time, i.e. the time available for O_2 diffusion (131, 132). During normoxic exercise the hyperpnoea-related increase in alveolar PO_2 generally accelerates trans-alveolar diffusion sufficiently to prevent a significant drop in arterial O_2 saturation (SaO_2) (17, 91). In contrast, capillary-mitochondrial diffusion is more sensitive to shortened transit times and may limit O_2 transport (106, 131), but probably only to a small extent (33, 109). Accordingly, the bottleneck is the convective process that connects the two sites of diffusive transport and as such, an individual's VO_2max is mainly determined by the capacity to increase cardiac output (17, 68, 109, 110). However, this order shifts in acute hypoxia where, despite an immediate reflex stimulation of pulmonary ventilation (139), alveolar PO_2 decreases (74, 93). Due to the passive nature of diffusive transport into the pulmonary capillaries arterial PO_2 diminishes in a direct response hereof. Furthermore, the narrower alveolar-capillary PO_2 gradient induces a growing pulmonary

diffusion limitation as cardiac output rises (25, 27, 54). The combination of these two factors leads to a progressive decrease in arterial O_2 content (c_aO_2) during incremental exercise. At submaximal workloads this is compensated for by an elevation of cardiac output which preserves O_2 delivery to the skeletal muscles (25, 37). However, as during maximal exercise neither cardiac output (110) nor the distribution of blood flow (25) are affected by acute hypoxia the lower c_aO_2 abates convective O_2 transport in direct proportion. This is further aggravated in hypoxia corresponding to altitudes > 4,500 m where maximal cardiac decreases in response to PO_2 (25, 125). Although the reason for this is not entirely clear it may account for approximately one third of the reduction in VO_2max in severe hypoxia (25). However, a contrasting viewpoint postulates that the lower cardiac output is not restrictive but rather a protective down-regulation that prevents excessive diffusion limitation at the pulmonary and muscular sites (135). As such it may prohibit increases in cardiac output that result in no gain or even a loss in mitochondrial O_2 supply.

Similar to normoxia it is controversial whether capillary-mitochondrial diffusion limits VO_2max in hypoxia (109, 132). A more important role is indicated as in severe hypoxia, an erythropoietin-induced elevation of c_aO_2 fails to improve VO_2max (98). This is in contrast to normoxia (78, 79, 98) or mild hypoxia (98) and may suggest that skeletal muscle O_2 uptake in severe hypoxia is limited by the capillary-mitochondrial diffusion gradient rather than by the convective transport rate. In contrast however, other studies have reported the relationship between convective O_2 supply and muscular O_2 consumption to be independent of PO_2 which argues against a major role of peripheral diffusion limitation also in hypoxia (25, 80).

In summary, VO_2max is determined by the O_2 supply to the mitochondria of the exercising muscle cells. In normoxia this is mainly confined by the capacity to increase cardiac output although a contribution of peripheral diffusion limitation cannot be ruled out. In hypoxia, the lower alveolar PO_2 and a resulting pulmonary diffusion limitation decrease arterial O_2 content and thus convective O_2 transport. As altitude exceeds 4,500 m, this is further exacerbated by a reduction in maximal cardiac output and potentially a capillary-mitochondrial diffusion limitation. All these factors together decrease mitochondrial O_2 supply and thus VO_2max.

Although the mechanisms introduced above are commonly accepted as the main reason for the limited aerobic exercise capacity in acute hypoxia, this does not exclude a potential contribution of more indirect factors. Particularly over the last decade growing evidence has suggested that the intrinsic response of the pulmonary circulation to acute hypoxia may provoke additional impairment. Furthermore, similarly recent studies have revealed a direct

impact of hypoxia on the central nervous system which may accelerate fatigue development independent of mitochondrial O_2 supply. The first two aims of the present PhD-thesis focussed on these mechanisms as specified in the following paragraphs.

Aim 1: Limitations induced by the pulmonary circulation

The pulmonary circulation responds to a decrease in alveolar PO_2 by hypoxic pulmonary vasoconstriction (HPV) (127) in the pre-capillary resistance vessels (119). This reflex leads to optimized ventilation/ perfusion matching by reducing blood flow to poorly ventilated alveoli (85). However, during hypoxic exposure PO_2 declines in all alveoli which induces a global instead of local vasoconstriction. As a results, pulmonary artery pressure (PAP) rises (86, 87) to an extent that is depending on the degree of hypoxia (53) and individual responsiveness (95) but potentially even beyond the threshold that defines severe pulmonary hypertension (87). Since in patients the latter induces exercise intolerance as a primary symptom (31, 137, 138) the HPV-induced rise in PAP might limit healthy humans by the same mechanisms: First, the blood flow resistance associated with the constricted pulmonary vasculature may diminish right ventricular stroke volume (89) which may translate into a reduced left ventricular diastolic filling and, by the Frank-Starling mechanism, constrain the capacity to increase cardiac output (90). Second, the elevated PAP may amplify arterial hypoxia by promoting ventilation/ perfusion mismatch as the global constriction of pulmonary resistance vessels impairs the ability to adjust blood flow to local differences in alveolar PO_2 (124, 128). Experimental evidence for a limiting role of the HPV was provided by several studies which attenuated PAP by pharmacological interventions (38-40, 48, 88, 97). The most frequently used agent for this purpose is the phosphodiesterase-5-inhibitor Sildenafil. This vasodilator has been reported to increase maximal cardiac output and exercise capacity in both, acute and chronic hypoxia, whereas SaO_2 was elevated in acute hypoxia only (48). As the latter did not seem to be a requirement for the performance gain it was concluded that the ergogenic action was rather based on right ventricular relief allowing for a higher cardiac output. Similar conclusions were drawn in two successive studies that applied endothelin receptor antagonists to reduce PAP (38, 88) which increased VO_2max without affecting SaO_2. In contrast, Faoro and colleagues observed a beneficial effect of Sildenafil only when an elevated SaO_2 was present at the same time and thus attributed it to changes in diffusive rather than convective O_2 transport (39). This was partially supported by another study that confirmed the positive effects of Sildenafil on SaO_2 and VO_2max whereas submaximal cardiac output remained

unchanged (97). Nevertheless, this could not exclude that the treatment affected cardiac output at higher workloads and thus may not rule out the limiting effect of right ventricular afterload that was suggested earlier. Taken together the ergogenic impact of pulmonary vasodilators in hypoxia indicates a limiting role of the HPV although the individual contribution of underlying mechanisms remains to date controversial.

Further specific evidence in this area was collected in subjects with a susceptibility to high altitude pulmonary edema (HAPE) (16). These persons are characterized by an excessive HPV that may cause pulmonary vessel leaking and the formation of extravascular fluid accumulation (15). Direct comparison between HAPE-susceptible and normal individuals therefore offers the possibility to examine the implications of different degrees of HPV independent of pharmacological vasodilation. Indeed, in a study that tested HAPE-susceptible subjects at 4,559 m the exercise limitation was larger than expected in normal individuals (73, 77) and attenuated by pulmonary vasodilation induced by the glucocorticoid Dexamethasone (40). However, although the pronounced exercise impairment is interesting it has to be interpreted with caution as comparisons between studies may be affected by different protocols and measurement techniques. Furthermore, as subjects were tested only a few hours after a strenuous two day ascent to 4,559 m exercise capacity might have been attenuated by preliminary fatigue (40). Finally, the trials were conducted in a supine position which may have in particular limited HAPE-susceptible subjects by spreading out subclinical pulmonary fluid accumulated in the basal region of the lung (114).

The first aim of this PhD-project was to confirm the previous observations in HAPE-susceptible individuals independent of these shortcomings. This was performed in two steps: First, by ensuring that the ergogenic effect of pulmonary vasodilation induced by Dexamethasone persists during a conventional cycle ergometer test and following a longer recovery period after the ascent to altitude. Second, by inclusion of normal individuals and direct comparison to the HAPE-susceptible subjects in the same study design.

The obtained results are presented in two manuscripts (114, 115) one of which (114) is to date published in a peer-reviewed journal whereas the other one will be submitted as a short report in the near future.

Aim 2: Limitations induced by the cerebral circulation

Strenuous exercise induces muscle fatigue that manifests by a reduction in maximal voluntary force generation (45). Our initial understanding of the factors leading to this phenomenon was largely based on the ideas of the English Nobelist Archibald Hill. He suggested that during fatiguing muscle work, a mismatch between O_2 demand and supply leads to biochemical perturbations within the myocytes that progressively constrain the shortening process (56-58). In accordance with this notion, performance in intense aerobic exercise tasks is to a large extent determined by the capacity to increase muscular O_2 supply (17, 68, 109, 110). However, a few years before Hill's hypothesis the Italian physiologist Angelo Mosso had introduced the concept that a central component, i.e. a process located within the brain, contributes to the development of muscle fatigue (84). Mosso interpreted this "central fatigue" as a protective mechanism that extorts abortion of exercise before muscular perturbation becomes excessive and may lead to tissue damage. Although this approach did initially not receive the same acceptance as Hill's model, it has attracted new interest as recent experiments provided evidence for central fatigue as indicated by a progressive decrease in motoneural drive despite a persisting requirement for force generation (46). Numerous studies have further indicated that the loss in voluntary force generation during fatiguing exercise is induced by the combination of a peripheral and a central component i.e. changes in the biochemical milieu within the myocytes (3, 4) and a reduced motoneural fibre recruitment (9). If the two components appeared independently they could easily be assessed by electromyography (EMG) as illustrated in figure 3 (49). However, as during exercise both components develop simultaneously, a more sophisticated approach needs to be applied. The most commonly used technique requires subjects to perform a maximal muscle contraction whereupon the motoneuron is magnetically stimulated (107). This generates supramaximal muscular fibre recruitment which results in a twitch-like increase of the maximal force output even in the unfatigued muscle (figure 4) (81). As the experiment is repeated after exercise a reduced force generation during supramaximal fibre recruitment illustrates the peripheral component of muscle fatigue (107). On the other hand, a larger difference between the voluntary and the magnetically induced supramaximal force output reveals the contribution of a diminished central motor drive, i.e. central fatigue. Such an experimental approach has repeatedly provided evidence for central fatigue to occur not only during resistance type exercise (14, 136) but also during strenuous endurance exercise (32, 67).

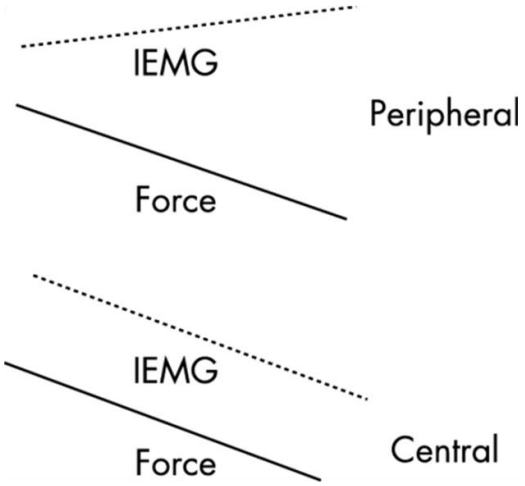

Figure 3: Integrated electromyogram (IEMG) and force output responses illustrating peripheral and central fatigue during exercise. With peripheral fatigue the force output decreases despite an unchanged or even increasing IEMG activity due to biochemical perturbations within the muscle fibre. In contrast, a parallel decline of IEMG and force output indicates undisturbed contractive function of the muscle fibres but a reduction in central motor drive, i.e. central fatigue. Reproduced from Gibson et al. 2004.

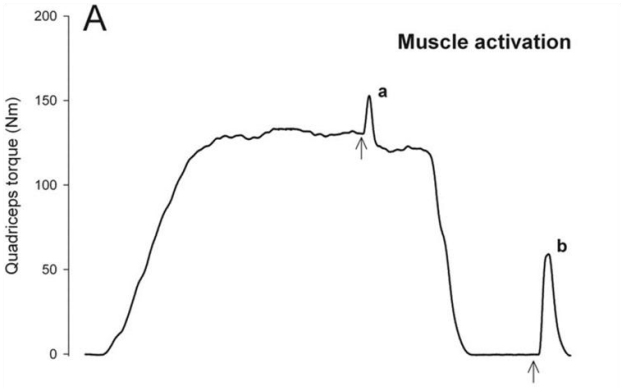

Figure 4: Muscular force development by supramaximal motoneural recruitment (a). This is induced by application of magnetic motoneural stimulation during a maximal voluntary contraction. (b) illustrates the effect of magnetic stimulation as the muscle is relaxed. Reproduced from Maffiuletti , 2010.

In line with Mosso's initial assumption central fatigue might indeed be a protective mechanism to prevent overstrain of the muscle fibres (84). Experiments during high-intensity cycling have revealed an individual threshold for peripheral fatigue development that could not be exceeded voluntarily (5, 9, 24). Instead, subjects subconsciously adapted their power output in time trials accordingly (11) whereas in fixed workload tests they exhausted as peripheral fatigue reached the critical threshold (9). The underlying regulation seems to depend on inhibitory feedback from metabosensitive muscle afferents that increase their discharge rate as fatigue related metabolites accumulate (6). This was experimentally demonstrated in subjects performing cycling time trials while the afferents from the locomotor muscles were pharmacologically blocked (7). Deprived from inhibitory feedback the central nervous system increased the motor drive which produced substantially more severe muscle fatigue than in the placebo trial. In fact, this study illustrated the importance of a protective role of central fatigue as, after the test, muscular exhaustion was so exaggerated that subjects temporarily experienced problems with simple muscular tasks such as walking or standing (7).

Although at first sight the role of O_2 appears minor in the process of central fatigue, the contrary is true, as hypoxia accelerates central fatigue development in different ways. First,

hypoxia increases the firing rate of the metabosensitive muscle afferents and thus the inhibitory feedback to the central nervous system (8, 10). This is the result of both, an increased resting discharge frequency, i.e. an effect on the nerves that is independent of exercise (60), and a faster accumulation of fatigue related metabolites during muscular work (72). Second, indirect evidence suggests an impeding influence of hypoxia on the supraspinal structures of the central nervous system (8, 121). Oxygenation of the cerebellum is determined by the product of arterial O_2 content and cerebral blood supply and both are negatively affected by hypoxia (64, 94): First, c_aO_2 is reduced which attenuates cerebral oxygenation if not compensated for by a higher blood supply (122). Second, arterial hypoxia stimulates peripheral chemoreceptors, causing reflex hyperpnoea (66) that partially restores arterial oxygenation (27) but also decreases arterial PCO_2 (125). As the latter is a potent regulator of cerebral artery vessel tone (63) hyperventilatory hypocapnia entails a reduction in cerebral blood supply (64, 122). Both mechanisms are further accelerated by exercise which decreases c_aO_2 (30, 44, 92) and induces further hyperventilation (66). The resulting decline in cerebral oxygenation might induce central fatigue independent of inhibitory muscle afferents (8, 10). This was suggested based on experiments where subjects performed exhaustive exercise in severe hypoxia. EMG recordings revealed that exhaustion was induced by a progressive decrease in central motor drive that occurred at a lower degree of peripheral fatigue than in normoxia (12, 121). However, as subjects were switched to hyperoxic breathing immediately before exhaustion cerebral oxygenation normalized within seconds which restored central neural drive and allowed subjects to continue exercising until the individual critical threshold in peripheral fatigue was reached. Nevertheless, it had to be acknowledged that hyperoxic breathing concomitantly restored oxygenation in the entire organism and that the individual effect of cerebral re-oxygenation could thus not be determined. Accordingly, Subudhi and colleagues attempted a specific re-oxygenation of the brain by enhancing cerebral blood flow with inspiratory CO_2 administration (123). This indeed elevated cerebral oxygenation as compared to sham treatment but, contrary to the hypothesis, did not increase VO_2max. However, the experiments were carried out in severe hypoxia corresponding to 4,875 m where a reduction in cardiac output (25) and peripheral O_2 diffusion limitations (98, 132) may have overruled a potential benefit of a restored cerebral oxygenation.

The second aim of this project was therefore to examine the effect of inspiratory CO_2 administration during exercise in more moderate hypoxia. We expected that in the absence of cardiac restrictions and potential peripheral diffusion limitations the increase in cerebral

oxygenation may increase VO_2max. The collected results are enclosed in a manuscript that is presently in press in a peer-reviewed journal (117).

1.3 Chronic hypoxia and aerobic exercise

As indicated, the exercise impairment in acute hypoxia is mainly the consequence of a reduced c_aO_2 that compromises convective O_2 transport. However, as exposure extends this is counteracted by an enhanced renal erythropoietin release (93, 96) which raises serum erythropoietin concentration to peak within 24-48 hours where after it slowly decreases to stabilize slightly above initial levels (1). The circulating erythropoietin progressively restores c_aO_2 by inducing polycythemia. This is initially entirely related to a contraction of plasma volume (118, 142) whereas erythropoiesis may increase the total volume of red blood cells (RCV) after several weeks (93, 96).

Although these haematological adaptations elevate c_aO_2 to eventually match or even exceed the values in normoxia the resulting impact on VO_2max is smaller than expected. Particularly in hypoxia corresponding to altitudes > 4,000 m acclimatization has little or no effect on VO_2max (18, 26, 28, 80) whereas in more moderate hypoxia the gain is larger (113). Similar observations were made in acute hypoxia if RCV was preliminarily increased by autologous blood transfusion (104, 105) or erythropoietin treatment (75, 98). This indicates that the normalization of c_aO_2 only benefits VO_2max in moderate hypoxia whereas other factors offset the ergogenic impact in severe hypoxia (26).

In contrast to these inconsistent consequences for VO_2max, the acclimatization process improves submaximal exercise capacity in all degrees of hypoxia (62, 82, 113). It is not entirely clear why the improvements persist in severe hypoxia despite an unchanged VO_2max but they might be related to a reduced perceived exertion during exercise as the increased c_aO_2 reduces the cardiac output required to still a given muscular O_2 demand.

The progressive restoration of submaximal exercise capacity is of high relevance for athletic performance at altitude and accordingly hypoxic exposure is a widespread preparative measure particularly in endurance sport. However, its application is not confined to the preparation for competitions at altitude as an erythropoietic elevation of the O_2 transport capacity is similarly ergogenic at sea level. This was clearly demonstrated following erythropoietin treatment (79, 98) or autologous blood transfusions (22, 36) whereas a reduction of RCV by phlebotomy has the contrary effect and reduces exercise capacity (36). As any manipulation of RCV is banned by the world anti-doping agency (WADA) endurance

athletes frequently attempt to stimulate erythropoiesis by hypoxia. This has led to the development of different forms of so called altitude training (76). The last aim of the present project was to examine the effects of the most contemporary altitude training strategy on highly trained endurance athletes, as explained below.

Aim 3: The effect of Live High-Train Low on elite athletes

The speculation that hypoxic exposure benefits sea level performance was initially inspired by the outstanding success of Eastern African runners who live and train at elevated altitude in their home countries (141). The classical altitude training concept attempted to copy these conditions and required athletes to spend several weeks of their preparation at moderate altitude (Live High – Train High, LHTH). However, despite the persisting popularity of LHTH its efficiency has always been subject to discord (41). Well-controlled studies in this field are scarce and although some of them observed an ergogenic effect (23, 83) others could not confirm this (2, 126). A potential explanation for the absence of performance gains following LHTH was provided by the findings of Levine and Stray-Gundersen (69). Although in their study LHTH elevated RCV and, in direct proportion, VO_2max of 13 sub-elite runners, this did not translate into a better 5 km running performance. According to the authors this may have been due to the impeding effect of even moderate hypoxia which lowered absolute training intensity during the LHTH period and may have led to a loss of muscular conditioning (69, 71). To avoid this process they introduced the Live High – Train Low (LHTL) strategy where athletes reside at similar altitude but train as close as possible to sea level to preserve training intensity (71). The applicability of LHTL was tested in the before mentioned study where another group of runners lived at 2,500 m but was transported to 1,300 m for daily training (69). This induced the same elevation of RCV and VO_2max as LHTH suggesting that the discontinuous hypoxic exposure was adequate to stimulate erythropoiesis. However, following LHTL these changes resulted in a better 5 km running performance presumably as training intensity could be preserved at the lower altitude. Similar findings were later obtained in some (21, 47, 103), but not all studies (100, 101), that induced the hypoxic stimulus by normobaric hypoxia in appropriate facilities. This modification became popular as it avoids the logistic effort of daily travelling and allows to conduct LHTL in countries that lack suitable training locations at altitude. Nevertheless, despite the promising findings of particularly the initial LHTL study (69) and the perspicuous rationale

for the superiority of this training strategy, the usefulness for endurance athletes is still debated due to several issues:

First, the efficiency of LHTL was never demonstrated in a placebo controlled study design. This is a serious concern as, even though the expected gains are substantial from an elite athlete's perspective (61), they are physiologically marginal (19, 52) and in the same range as a placebo-effect may enhance endurance performance (29). Given the considerable financial costs of appropriate facilities as well as the time exposure and social constraints associated for athletes it is inevitable to confirm that LHTL is not just an expensive and inconvenient placebo treatment.

Second, the impact of LHTL has barely been examined in true elite athletes. As indicated, expected performance gains are minor and thus this training form seems not relevant for sub-elite athletes who preserve the capacity to improve by conventional measures. In contrast, elite athletes have often reached the limit of physiological adaptability to normal training stimuli (50, 99). As in these individuals even the slightest difference may determine between winning and losing (61) they are the appropriate target group for LHTL and studies should include subjects accordingly.

Finally, beyond the pivotal discord about the efficiency of LHTL is a widespread disagreement regarding the underlying mechanisms (52, 70). The most popular explanation is the erythropoietic approach which attributes performance gains to an optimized convective O_2 transport capacity (69, 70). Nevertheless, this is solely based on correlative analyses and theoretical reasoning and lacks direct evidence. Furthermore, different studies have reported LHTL to benefit endurance performance independent of changes in RCV but by improving exercise economy (51, 112). Such an adaptation allows athletes to race at the same perceived intensity while producing a higher speed which is an enormous advantage (65). Similar to other potential effects of LHTL, however, an improved exercise economy is not a consistent finding and lacks confirmation from a placebo-controlled study.

The third and final aim of this project was to resolve these limitations of previous LHTL studies by conducting a placebo-controlled trial including elite athletes as subjects. Furthermore specific experimental interventions were included to establish the mechanisms that underlie potential performance gains. The observations made in this study are presented in two manuscripts that are published in peer-reviewed journals (102, 116).

2. Manuscripts

2.1 Dexamethasone Improves Maximal Exercise Capacity of Individuals Susceptible to High Altitude Pulmonary Edema at 4559m

2.2 Maximal exercise capacity in individuals susceptible to high altitude pulmonary edema at 4559 m

2.3 Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O_2 uptake

2.4 "Live high–train low" using normobaric hypoxia: a double-blinded, placebo-controlled study

2.5 The role of haemoglobin mass on VO_2max following normobaric 'live high-train low' in endurance-trained athletes

HIGH ALTITUDE MEDICINE & BIOLOGY
Volume 12, Number 2, 2011

DOI: 10.1089/ham.2010.1075

Dexamethasone Improves Maximal Exercise Capacity of Individuals Susceptible to High Altitude Pulmonary Edema at 4559 m

Christoph Siebenmann,[1,2] Konrad E. Bloch,[2,3] Carsten Lundby,[2] Yvonne Nussbamer-Ochsner,[3] Michèle Schoeb,[4] and Marco Maggiorini[4]

Abstract

Siebenmann, Christoph, Bloch, Konrad E., Lundby, Carsten, Yvonne Nussbaumer-Ochsner, Michèle Schoeb, and Marco Maggiorini. Dexamethasone improves maximal excercise capacity of individuals susceptible to high altitude pulmonary edema at 4559 m. *High Alt. Med. Biol.* 12:169–177, 2011.—We have previously demonstrated that prophylactic intake of dexamethasone improves maximal oxygen uptake (Vo_2max) in high altitude pulmonary edema (HAPE) susceptible subjects 4 to 6 h after a 2-day climb to 4559 m. However, since with this ascent protocol HAPE usually develops after the first night at 4559 m or later, we hypothesized that a continued dexamethasone prophylaxis would result in an even more pronounced improvement of Vo_2max after an additional night at high altitude.

Vo_2max of 24 HAPE susceptibles was evaluated on a bicycle ergometer at an altitude of 490 m and at 24 h after rapid ascent to 4559 m. Subjects were divided into two groups: The control group ($n = 14$) performed both tests without dexamethasone, whereas the dexamethasone group ($n = 10$) received dexamethasone 8 mg twice a day (b.i.d), starting 24 h prior to ascent.

At 4559 m, Vo_2max was $61\% \pm 6\%$ of the baseline value in the control group and $70\% \pm 9\%$ in the dexamethasone group ($p = 0.025$). Similarly, O_2 pulse (Vo_2/heart rate) was $68\% \pm 7\%$ and $77\% \pm 11\%$ of baseline, respectively ($p = 0.043$). Arterial O_2 saturation at maximal exercise did not differ between groups, whereas at rest it was $83\% \pm 10\%$ in the control group and $91\% \pm 4\%$ in the dexamethasone group ($p = 0.009$).

Dexamethasone prophylaxis increased Vo_2max of HAPE-susceptible individuals after the first night at 4559 m without affecting arterial O_2 saturation at maximal exercise. This might be explained by a sustained effect of dexamethasone on maximal cardiac output and pulmonary O_2 diffusion, both resulting in enhanced convectional O_2 transport to the locomotor muscles.

Key Words: hypoxia; Vo_2max; pulmonary circulation

Introduction

Maximal O_2 uptake (Vo_2max) is reduced in acute hypoxia (Wagner, 2000; Calbet and Lundby, 2009) owing to two main mechanisms (Calbet et al., 2003): First, the arterial Po_2 (Pao_2) is decreased due to both a lower alveolar Po_2 and a resulting pulmonary diffusion limitation for O_2 (Wagner, 2000; Calbet et al., 2003; Lundby et al., 2006). Second, maximal cardiac output is reduced in severe hypoxia owing to a decrease in stroke volume and maximal heart rate (HR) (Calbet et al., 2003). Each mechanism contributes to a lower O_2 delivery to the locomotor muscles and therefore a decline in Vo_2max.

The decline in Vo_2max might be even more prominent in persons who develop a subclinical (Cremona et al., 2002) or overt high altitude pulmonary edema (HAPE) (Bartsch et al., 2003). HAPE is a noncardiogenic edema, typically occurring after rapid ascent to altitudes higher than 2500 m (Bartsch

[1]Institute of Human Movement Sciences and Sport, ETH, Zurich, Switzerland.
[2]Center for Integrative Human Physiology (ZIHP), Institute of Physiology, University of Zurich, Zurich, Switzerland.
[3]Pulmonary Division, University Hospital of Zurich, Zurich, Switzerland.
[4]Intensive Care Unit DIM, University Hospital of Zurich, Zurich, Switzerland.

et al., 2003). In individuals susceptible to HAPE (HAPE-s), the mechanism of HAPE pathogenesis is an excessive and inhomogeneous increase in pulmonary artery resistance because of vasoconstriction in response to acute hypoxia (Bartsch et al., 2005). This leads to overperfusion of remaining patent vessels and subsequent injury of pulmonary capillary walls, with leakage of red blood cells and proteins into the airways and alveoli (Hultgren, 1996). Although HAPE is a life-threatening and rare disease, subclinical pulmonary extravascular fluid accumulation is more prevalent and may be present in about 75% of non-HAPE-s mountaineers examined shortly after arrival at an altitude of 4559 m (Cremona et al., 2002).

The pathogenesis of HAPE may potentiate the Vo_2max-reducing factors in acute hypoxia. Interstitial pulmonary edema elongates the diffusion distance of O_2 from the alveoli into the pulmonary capillaries (Steinacker et al., 1998) and negatively influences ventilation–perfusion matching, thereby deteriorating respiratory efficiency during heavy exercise (Podolsky et al., 1996). All these factors result in a more prominent decrease in Pao_2. In addition, excessive elevations in pulmonary arterial resistance could increase the afterload of the right ventricle and reduce its stroke volume, thereby decreasing left ventricular preload and maximal cardiac output (Ghofrani et al., 2004).

An efficient intervention to avoid health threat in climbers and travelers that do not have the opportunity to acclimatize before an ascent to altitudes higher than 2500 m is the prophylactic administration of dexamethasone, a synthetic glucocorticoid. Dexamethasone is known to be effective as a prophylaxis against acute mountain sickness (AMS) (Johnson et al., 1984) and even superior to the broadly used carbonic anhydrase inhibitor acetazolamide (Ellsworth et al., 1991). If treatment is started prior to exposure to hypoxia, dexamethasone prophylaxis decreases pulmonary arterial resistance (Maggiorini et al., 2006) and enhances alveolar fluid clearance (Folkesson et al., 2000; Noda et al., 2003; Guney et al., 2007). Both mechanisms contribute to a lower incidence of HAPE. Dexamethasone was also found to increase resting Pao_2 in acute hypoxia (Maggiorini et al., 2006).

The effect of dexamethasone on HAPE-s has been broadly examined in resting conditions; however, the influences on exercise performance are poorly explored. Fischler and colleagues (Fischler et al., 2009) reported that dexamethasone taken before a 2-day ascent to the Capanna Regina Margherita research facility (Italy, 4559 m) increased the Vo_2max of HAPE-s subjects measured 4 to 6 h after arrival. However, since HAPE normally develops after 2 to 5 days of hypoxic exposure (Bartsch et al., 2005) and, in our experience with this ascent profile, usually after the first night at the Margherita hut or later, the adverse impact of HAPE pathogenesis on Vo_2max may be more pronounced after a longer period of hypoxic exposure, which may enhance the beneficial effect of dexamethasone. We therefore tested the hypothesis that the benefit of a dexamethasone prophylaxis on Vo_2max would be maintained and more pronounced in HAPE-s subjects after staying an additional night at the Margherita hut.

Methods

The current study includes 16 of 25 HAPE-s mountaineers who initially participated in a randomized, double-blind, placebo-controlled study in 2007. Because of 9 dropouts due to bad weather conditions and incapacitating acute mountain sickness (AMS) before arriving at 4559 m, 8 additional HAPE-s mountaineers were recruited according to the same inclusion criteria in a complementary open-label study in 2009. Both studies followed similar protocols and were approved by the Ethical Committee of the University of Zurich and conformed to the Declaration of Helsinki. Subjects gave written informed consent to participation.

Subjects

In total, 24 subjects were finally included into the present analysis. Criteria of inclusion was susceptibility to HAPE, which was defined by a history of radiologically or clinically diagnosed HAPE and confirmed in an interview by a physician with long-time experience in high altitude medicine. Criteria of exclusion included age below 18 and above 65 yr, chronic intake of medication, or previous diagnosis of cardiopulmonary and other chronic diseases. Further, we excluded mountaineers who had spent more than 5 nights at an altitude higher than 2500 m within the last 30 days of the beginning of the investigation. Anthropometric data of the subjects included in the study are summarized in Table 1.

Protocol

Baseline examinations were conducted at the University Hospital of Zurich (490 m). Subjects spent 2 days in the hospital performing a baseline cardiopulmonary exercise test (CPET) in the late afternoon (2007) or during the course of the morning (2009). In both settings, CPET was performed at least 2 h after the last meal.

Two to three weeks after baseline measurements, all study participants traveled to Alagna (Italy, 1205 m) in groups of 2 to 4. From there they were carried by cable car to an altitude of 2900 m. They continued by foot to the Gnifetti hut (3647 m), where they arrived in the late afternoon and spent the night. The following morning they reached the Margherita hut (4559 m) after 4 to 6 h of ascent. Throughout the entire trip, all subjects were accompanied by a professional mountain guide. CPET was performed the morning following night 1 spent at the Margherita hut. Subjects then stayed at the hut 3 additional consecutive nights for further research and climbed back down in the morning of day 5.

Medication

The 16 subjects from the 2007 study were randomized to receive either 8 mg dexamethasone b.i.d (Fortecortin, Merck,

Table 1. Anthropometric Data of the Subjects

	Control group	*Dexamethasone group*	p-*values*
Number of subjects	14	10	
Male	9	8	
Female	5	2	
Age (years)	45 ± 8.6	47 ± 9.6	0.51
Height (m)	1.74 ± 0.08	1.72 ± 0.05	0.34
Weight (kg)	70.2 ± 7.2	75.7 ± 12.9	0.31
BMI (kg/m^2)	23.1 ± 1.5	25.5 ± 3.7	0.15

Control group = untreated group; Dexamethasone group = Dexamethasone-treated group; BMI = Body mass index.

Whitehouse, NJ, USA) (dexamethasone group, $n = 10$) or placebo (control group, $n = 6$) during their stay at high altitude, with treatment beginning 24 h prior to ascent. They started intake autonomously, but the remaining number of pills and further intake were controlled at the Margherita hut. Dexamethasone and placebo were packed into identical white capsules so that neither subjects nor investigators could distinguish between them. However, open-labeled emergency treatment with dexamethasone was always available for subjects of the control group presenting with severe AMS.

The 8 additional subjects included in the 2009 open-label study to increase the number of participants within the control group (final $n = 14$) did not receive placebo or dexamethasone before CPET. As in the 2007 study, dexamethasone was provided as emergency treatment. However, in both years, no participant randomized to the control group required emergency treatment with dexamethasone before CPET.

Assessment of AMS and HAPE

In the morning before CPET, AMS was assessed by the Lake Louise protocol, which consists of an interview and a clinical examination (for details, see Maggiorini et al., 1998). A score >4 was used as an indicator for AMS. Further, subjects were regularly examined for symptoms and signs of HAPE. These examinations included daily auscultation of the lungs and chest radiography on the second day at the Margherita hut.

Conduction of CPET

CPET was performed on an electronically braked bicycle ergometer in an upright position (Cyclus 2, RBM Elektronik, Leibzig, Germany). Subjects wore a face mask that covered the mouth and nose for complete breath collection. Amperometric solid-state electrolyte sensors continuously measured O_2 and CO_2 concentration in the expired gas. Results were monitored and saved as breath-by-breath values on a portable computer. The utilized system (ZAN 600 USB, nSpire Health, Louisville, KY, USA) was calibrated immediately before each test. HR was detected by electrocardiogram (CardioCollect 12, Spacelabs Healthcare, Feucht, Germany), and peripheral arterial O_2 saturation (Spo_2) was measured by pulse oximetry (Masimo SET Radical, Inspiration Medical, Bochum, Germany) on the subject's earlobe. Estimated maximal voluntary ventilation (MVV) was calculated as forced expiratory volume in 1 sec (FEV_1)×40 (ACCP, 2003).

Resting values were obtained with the subjects sitting still on the bicycle for 2 min. They then started exercising at a workload (W) of 50 W that was increased by 10 to 40 W/ min according to a progressive ramp protocol individually tailored to lead to exhaustion within 8 to 12 min (ACCP, 2003). To maintain test duration, increases in work rate were approximately 30% lower at 4559 m than at baseline (490 m). During the last minutes, subjects were vigorously motivated to continue until maximal exhaustion. All subjects reached maximal exhaustion according to standard criteria (ACCP, 2003).

Respiratory parameters of 2 subjects of the control group could not be properly measured because of technical difficulties while at 4559 m; therefore, these parameters, along with the corresponding low-altitude values, were discarded.

Statistical analysis

Data were analyzed using Statistica 6.0 (Statsoft, USA) To evaluate differences between the two groups, the values at high altitude were compared in percents of the values at low altitude using a Mann–Whitney *U* test for nonparametric and independent samples. The effect of the exposure to high altitude within each group was evaluated by the Wilcoxon matched pairs test. Results are given as mean ± SD; a p value of <0.05 was considered statistically significant.

Results

Baseline testing

Baseline examinations at 490 m revealed that subjects were healthy, with normal pulmonary functions and free of signs of pulmonary hypertension. None were on any medications or supplements that might interfere with our experiments. All were able and motivated to perform CPET to complete exhaustion (Table 2).

Altitude exposure

Exposure to hypoxia was well tolerated by all subjects, and they were able to perform the exercise trials (Table 2). Nevertheless, Lake Louise scores evaluated in the morning before CPET were 5.71 ± 2.55 in the control group and 3.70 ± 1.95 in the dexamethasone-group ($p = 0.036$); a Lake Louise score >4, indicating the presence of AMS, was found in 9 controls, but in only 3 subjects of the dexamethasone group. No signs of HAPE were observed in either group on the first 2 days at the Margherita hut.

Resting HR was increased by 26% in the control group and reduced by 2% in the dexamethasone group ($p = 0.007$) from baseline values, while resting Spo_2 was 83% ± 10% in the control group and 91 ± 4% in the dexamethasone group, respectively ($p = 0.009$). Table 2 summarizes the CPET results for both conditions and illustrates the influence of the rapid ascent to 4559 m on exercise capacity. In both groups, hypoxia induced a substantial decrease in Vo_2max. Nevertheless, the respiratory exchange ratio ($RER = Vco_2/Vo_2$) was similar to baseline in both groups, indicating maximal effort at high altitude despite the presence of AMS, especially in the control group, and no significant difference in RER between groups was observed. Further, no correlation was found between Lake Louise scores and RER ($R^2 = 0.06$).

Effects of dexamethasone prophylaxis on maximal exercise capacity

To evaluate the influence of the dexamethasone prophylaxis on maximal exercise capacity, we compared the results achieved at 4559 m in percents of the baseline values (Table 3). Compared with the control group, the decrease in maximal workload (Wmax) was 7% smaller in the dexamethasone group ($p = 0.004$). This was accompanied by an almost 10% smaller decrease in Vo_2max in the dexamethasone group ($p = 0.025$). Despite these differences, minute ventilation (V_E) was similar between groups and to values obtained at low altitude, as was V_E/Vo_2. However, V_E/Vco_2 at high altitude was increased in both groups, whereas the increase was

Table 2. Cardiorespiratory Parameters During Maximal Exercise at 490 m and 4559 m

	Zurich, 490m		*Margherita, Day 2, 4,559 m*		p-*value for altitude effect*	
Group	*Control group*	*Dexamethasone group*	*Control group*	*Dexamethasone group*	*Control group*	*Dexamethasone group*
Vo_2max [L/min]	3.58 ± 0.69	3.59 ± 0.78	2.17 ± 0.43	2.47 ± 0.44	0.002	0.005
Vco_2 [L/min]	4.15 ± 0.82	4.18 ± 0.89	2.44 ± 0.55	2.93 ± 0.57	0.002	0.005
Wmax [W]	279 ± 68	300 ± 58	178 ± 51	213 ± 50	0.001	0.005
HR [1/min]	179 ± 8	179 ± 14	164 ± 12	163 ± 13	0.002	0.008
V_E [L/min]	131 ± 22.7	131 ± 22.8	129 ± 28.9	137 ± 26	0.754	0.241
(% max)	(85)	(91)	(93)	(102)		
MVV [L/min]	154 ± 21.4	145 ± 21.1	138 ± 22.2	136 ± 23.1	0.002	0.007
V_E/Vo_2	37.1 ± 5.2	37.0 ± 5.3	59.3 ± 8.0	56.0 ± 7.7	0.002	0.005
V_E/Vco_2	32.0 ± 4.2	31.8 ± 4.6	53.1 ± 7.0	47.3 ± 6.9	0.002	0.005
F [1/min]	45.8 ± 5.4	47.1 ± 7.5	50.4 ± 9.8	50.8 ± 9.2	0.060	0.074
V_T [L]	2.87 ± 0.43	2.83 ± 0.60	2.58 ± 0.49	2.74 ± 0.51	0.002	0.445
F/V_T [1/[L*min]]	16.3 ± 3.6	17.9 ± 6.6	20.4 ± 6.4	19.5 ± 6.7	0.005	0.093
RER	1.16 ± 0.04	1.16 ± 0.05	1.12 ± 0.07	1.19 ± 0.07	0.084	0.333
Spo_2 [%]	96.1 ± 2.7	97.6 ± 2.2	72.6 ± 14.2	74.0 ± 8.98	0.001	0.005

Control-group = untreated group; Dexamethasone-group = Dexamethasone-treated group; Vo_2max = maximal O_2 uptake; $Vco_2 = CO_2$ output; Wmax = maximal workload; HR = heart rate; V_E = minute ventilation; MVV = calculated maximal voluntary ventilation; F = respiratory frequency; V_T = tidal volume; RER = respiratory exchange ratio (Vco_2/Vo_2); Spo_2 = arterial O_2 Saturation.

almost 18% higher in the control group than in the dexamethasone group ($p = 0.0001$). Tidal volume (V_T) tended to be larger in the dexamethasone group ($p = 0.050$) in group comparison, whereas it was reduced at high altitude in the control group only.

At maximal exercise, neither HR nor Spo_2 differed between the groups. O_2 pulse (Vo_2/HR), an indirect measurement of stroke volume (ACCP, 2003), was 67.7% ± 6.5% of the baseline value in the control group and 76.6% ± 11.2% in the dexamethasone group ($p = 0.043$). Individual responses in Wmax, Vo_2max and Spo_2 to the hypoxic exposure are illustrated in Fig. 1.

Figure 2 depicts the correlation between Vo_2max and Spo_2 at 4559 m, both expressed as percentages of the values at low altitudes. A strong and significant correlation was present in the dexamethasone group, but not in the control group.

Table 3. Cardiorespiratory Parameters During Maximal Exercise at 4559 m in Percents of Baseline Values

	Control group	*Dexamethasone group*	p-*value*
Vo_2max [%]	60.9 ± 5.7	69.7 ± 8.6	0.025
Vco_2 [%]	58.9 ± 7.1	71.0 ± 8.4	0.003
Wmax [%]	63.4 ± 6.2	70.5 ± 4.3	0.004
HR [%]	91.7 ± 5.9	91.4 ± 6.3	0.98
V_E [%]	97.6 ± 10.0	105.9 ± 16.0	0.16
V_E/Vo_2 [%]	160.5 ± 11.0	151.8 ± 11.2	0.16
V_E/Vco_2 [%]	166.4 ± 11.6	148.8 ± 7.9	0.0001
F [%]	109.7 ± 14.0	108.0 ± 12.9	0.77
V_T [%]	89.6 ± 8.2	98.1 ± 9.7	0.05
F/V_T [%]	124.3 ± 25.8	111.1 ± 18.4	0.14
RER [%]	96.6 ± 6.6	102.0 ± 5.3	0.06
Spo_2 [%]	75.5 ± 13.6	75.7 ± 7.9	0.58

Control group = untreated group; Dexamethasone group = Dexamethasone-treated group; Vo_2max = maximal O_2 uptake; $Vco_2 = CO_2$ output; Wmax = maximal workload; HR = heart rate; V_E = minute ventilation; MVV = calculated maximal voluntary ventilation; F = respiratory frequency; V_T = tidal volume; RER = respiratory exchange ratio (Vco_2/Vo_2); Spo_2 = arterial O_2 Saturation.

Effects of the placebo treatment

To establish the effect of the placebo treatment that was applied in 2007, but not in 2009, to subjects of the control group, the hypoxic Vo_2max of these two subgroups was compared. In the placebo-treated control-group subjects of 2007, Vo_2max was 60% ± 7% of the baseline value, whereas control-group subjects of 2009 that did not receive placebo obtained 62% ± 4% ($p = 0.7$). Further, Vo_2max in both subgroups was significantly lower than in the dexamethasone group.

Discussion

The purpose of the present study was to test the hypothesis that a prophylactic oral administration of dexamethasone 8 mg b.i.d, starting 24 h prior to ascent and maintained throughout an overnight stay at 4559 m, provides persistent increases in hypoxic Vo_2max in HAPE-s subjects. Our major finding is that dexamethasone significantly reduced the hypoxia-related decline in Vo_2max and resting Spo_2. Further, symptoms of AMS were significantly abated in the dexamethasone group.

Our results confirm and extend a previous study that reported an improvement of Vo_2max after rapid ascent to 4559 m by prophylactic dexamethasone administration (Fischler et al., 2009). However, in the earlier investigation, CPET was conducted only 4 to 6 h after arrival at 4559 m and thus several hours or days before development of HAPE generally occurs (Bartsch et al., 2005). To evaluate whether continued administration of dexamethasone would provide persistent and more distinct improvements of Vo_2max, the present results were collected following an additional 24 h of hypoxic exposure. Indeed, in the earlier study, Vo_2max of untreated subjects decreased by 52% from values at low altitude, and dexamethasone prophylaxis reduced this decrease

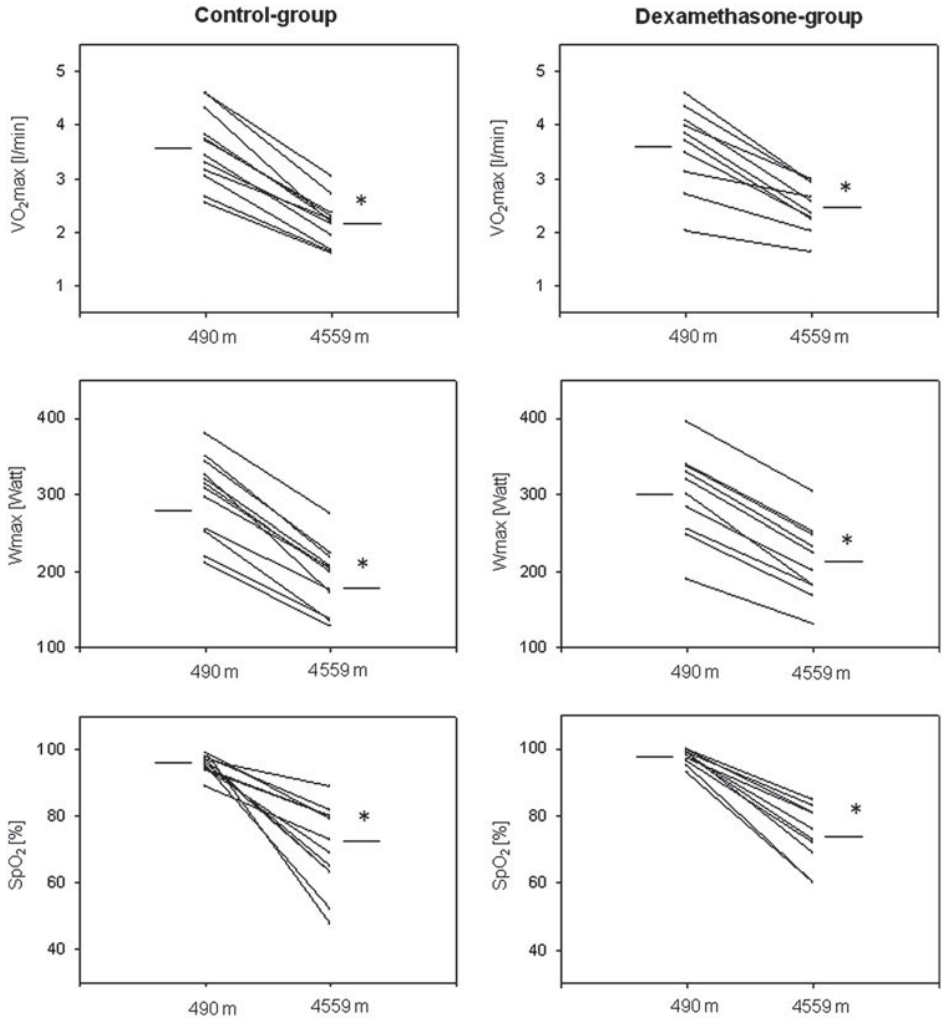

FIG. 1. Effect of acute hypoxia on treated and untreated subjects. Control group, untreated group; dexamethasone group, dexamethasone-treated group; Vo_2max, maximal O_2 uptake; Wmax, maximal workload; Spo_2, arterial O_2 saturation during maximal exercise; horizontal lines represent mean values at both altitudes; $^*p < 0.05$ vs. mean value at 490 m.

by 7% (Fischler et al., 2009). The present study shows that dexamethasone reduced the 39% decrease in the control group subjects by 9%. Furthermore, in the earlier study (Fischler et al., 2009), dexamethasone treatment improved only Vo_2max, but not Wmax, which is surprising, because acute hypoxia has been shown not to alter external work efficiency (Lundby et al., 2007). In contrast, we observed dexamethasone to improve Wmax and Vo_2max similarly, which is in accordance with an unchanged work efficiency (Vo_2/W relationship) in hypoxia. Our observations further extend the data by Fischler and colleagues (2009); they tested their subjects in a very unusual semirecumbent exercise position, whereas we demonstrate beneficial effects of dexamethasone on Vo_2max in a more familiar upright body position.

Generally, rapid ascent to high altitude is associated with an impairment of Vo_2max that becomes more severe with increasing altitude. According to Fulco and colleagues (1998), the expected decrease at 4559 m compared with 490 m is about 30%. In accordance with this, previous observations on Vo_2max at the Margherita hut in non-HAPE-s subjects report an average decrease of 31% (Lundby, 2008) or 21% (Lundby et al., 2001). In the current study, rapid ascent led to a reduction of 39% in the control group. Although comparisons between studies should be interpreted cautiously since different protocols and measurement techniques may influence the outcomes, our results indicate a rather marked deterioration in exercise capacity in untreated HAPE-s subjects compared with normal individuals. This is in line with previous findings (Fischler et al., 2009) that reported an even larger impairment, with untreated HAPE-s persons decreasing by 52%. The additional impairment in that study may be explained by the semirecumbent exercise position, which reduces Vo_2max in healthy subjects at sea level (Pedersen et al., 1996) and might additionally impair exercise in HAPE-s subjects at high altitude by spreading extravascular fluid, originally trapped by gravity in the basal region of the lung, over a larger pulmonary area. Further, CPET in the earlier study was performed only a few hours after the subjects' arrival at the Margherita hut. The strenuous ascent on the testing day may have further contributed to the larger decrease in maximal exercise capacity by fatiguing the subjects (Fischler et al., 2009).

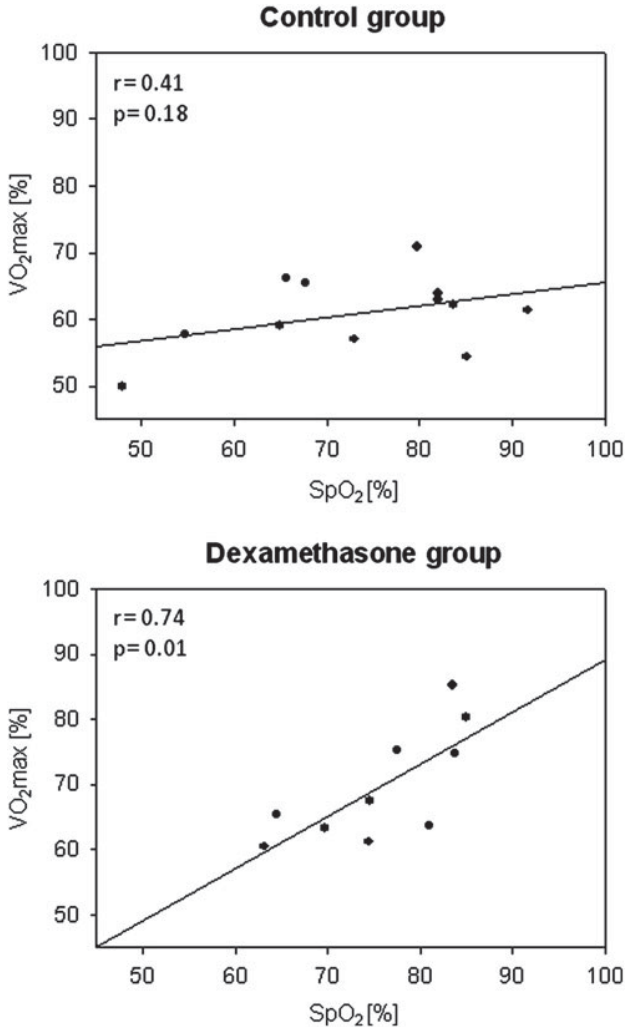

FIG. 2. Correlation between arterial O_2 saturation and maximal O_2 uptake at 4559 m. Control group, untreated group; dexamethasone group, dexamethasone-treated group; Vo_2max, maximal O_2 uptake expressed in percent of value at low altitude; Spo_2, arterial O_2 saturation during maximal exercise expressed in percent of value at low altitude.

What are the mechanisms by which dexamethasone induces improvements in Vo_2max in HAPE-s individuals? During acute exposure to altitudes higher than 4000 m, Vo_2max is mainly decreased by reductions in arterial O_2 content and in maximal cardiac output, both contributing to an attenuation in convective O_2 transport to the locomotor muscles (Calbet et al., 2003). The dexamethasone prophylaxis may beneficially influence both of these factors: in acute hypoxia, dexamethasone acts as a vasodilator that abates the exaggerated rise in pulmonary artery pressure that is characteristic for the development of HAPE (Maggiorini et al., 2006). Increases in hypoxic Vo_2max with pulmonary vasodilatation have been reported previously in studies that induced vasodilatation with the phosphodiesterase-5 inhibitor sildenafil (Ghofrani et al., 2004; Richalet et al., 2005; Faoro et al., 2007). In these studies, the performance-enhancing effect was explained by either a reduction in right ventricular afterload, resulting in higher cardiac output (Ghofrani et al., 2004), an improved arterial oxygenation (Faoro et al., 2007), or both (Richalet et al., 2005).

In the present data, O_2 pulse is higher in the dexamethasone group than in the control group despite similar maximal HR and Spo_2. This suggests that the dexamethasone prophylaxis led to an increase in maximal cardiac output, which may enhance peripheral O_2 delivery and therefore Vo_2max. Although there is some concern about using O_2 pulse as an approximation for stroke volume during exercise in hypoxic environments owing to arterial O_2 desaturation (ACCP, 2003), we argue that the estimation remains applicable in our data, because exercise Spo_2 is very similar in both groups, indicating a fairly equal amount of transported O_2 in a given volume of blood. Of interest, a similar exercise Spo_2 was also reported previously (Fischler et al., 2009) and is observed despite the higher levels Vo_2max and likely cardiac output that may shorten pulmonary transit time and promote pulmonary diffusion limitation in the dexamethasone group (Hopkins et al., 1996). Therefore, the lack of a difference in exercise Spo_2 between subject groups in the present study indicates an enhanced pulmonary O_2 diffusion in the dexamethasone group, allowing for Spo_2 to obtain similar levels as in the controls despite higher exercise intensities, an explanation that is supported by the finding of higher resting Spo_2 in the dexamethasone group. An improved pulmonary O_2 diffusion in subjects receiving dexamethasone may have emerged from either an optimized blood distribution over the pulmonary vessels, because subclinical HAPE has been reported to promote ventilation–perfusion inequalities (Podolsky et al., 1996), or from an elevated transpulmonary O_2 diffusion, resulting from a reduction in pulmonary extravascular fluid accumulation (Steinacker et al., 1998). These explanations are supported by the lower V_E/Vco_2 in the dexamethasone group, which indicates a better ventilatory efficiency with dexamethasone. Thus, the improvement in Vo_2max in the dexamethasone group may be explained by a combination of both, an increase in maximal cardiac output, and an improvement in pulmonary O_2 diffusion. In favor of this explanation, we observed that Vo_2max and Spo_2 were significantly correlated only in the dexamethasone group. A hampered right ventricular performance with excessive afterload may have caused exhaustion in the control group before Spo_2 became a limiting factor.

However, the question about the mechanism by which dexamethasone may increase maximal cardiac output remains debatable. As suggested in several similar studies applying different pulmonary vasodilators (Ghofrani et al., 2004; Richalet et al., 2005; Faoro et al., 2009), a decrease in right ventricular afterload may explain higher levels of cardiac output. In turn, it has been proposed that the reduction in maximal cardiac output in hypoxia may result from a cardiac downregulation aiming to prevent an excessive widening of the alveolar–arterial Po_2 difference with decreasing pulmonary transit times (Calbet et al., 2003). Therefore, an improved pulmonary O_2 diffusion may allow for cardiac output to obtain higher levels before reaching the point where further increases would result in no or even negative changes in peripheral O_2 delivery. This explanation is supported by the finding that Spo_2 was higher in the dexamethasone group at rest, but did not differ between groups at maximal exercise where a potential downregulation of cardiac output may have prevented further desaturation. Nevertheless, in a recent study the effect of the dual endothelin receptor antagonist

bosentan on hypoxic Vo_2max was investigated (Faoro et al., 2009). It was demonstrated that bosentan significantly lowers pulmonary artery pressures and concomitantly improves hypoxic Vo_2max without affecting Spo_2. Therefore, the conclusion was drawn that right ventricular afterload reduction is a key to improve exercise capacity at high altitude. Taken together, these findings indicate that the cause or effect question concerning increased cardiac output with improved pulmonary O_2 diffusion remains controversial and requires further investigation with a reliable assessment of cardiac output.

We also observed an increase in resting HR at 4559 m only in the control group, whereas in those participants receiving dexamethasone, resting HR was similar to baseline values. This is surprising, because acute exposure to hypoxia is normally accompanied by higher resting HR (Vogel and Harris, 1967). Nevertheless, a blunted HR after intake of dexamethasone in acute hypoxia has been reported previously and may be related to a modulation of increased sympathetic drive (Maggiorini et al., 2006), which is a potential contributor to increased pulmonary vascular resistance and pulmonary capillary permeability (Duplain et al., 1999). However, in agreement with earlier observations (Fischler et al., 2009), acute hypoxia decreased maximal HR to a very similar degree in both groups. Consequently, there is a larger difference between resting and maximal HR; thus, an increased HR reserve in the dexamethasone group may have contributed to the gain in maximal exercise capacity and supports the explanation of right ventricular unloading in dexamethasone-treated subjects.

It is important to note that dexamethasone might improve exercise capacity even in normoxic conditions: high dosages have been used in endurance sports on various occasions to enhance performance (Arlettaz et al., 2006). Therefore, the World Anti-Doping Agency (WADA) prohibits administration of glucocorticosteroids during competitions. This raises the question of whether our finding is at all hypoxia related or just a consequence of a general performance-enhancing effect of dexamethasone that is also present in normoxic conditions. However, several studies that have investigated the effect of dexamethasone on normoxic exercise capacity found no improvement in submaximal (Viru and Smirnova, 1982; Arlettaz et al., 2006; Cordova Martinez, 2006; Arlettaz et al., 2008a) or maximal (Marquet et al., 1999; Cordova Martinez, 2006; Arlettaz et al., 2008b) exercise capacity, suggesting that the amelioration that dexamethasone brought to our subjects was indeed owing to a reduction of hypoxia-related impairment of exercise tolerance.

Limitations

We tested our hypothesis by comparing a treatment group to untreated subjects. The optimal design would have been a crossover protocol in which each subject is its own control. However, the obvious inconvenience for the subjects of traveling to the Margherita hut twice argued against this design. Further, the scheduling of the second ascent would have been problematic. A short interim would have allowed for an altitude acclimatization carry-over. On the other hand, with a long break, other factors, such as altered physical conditioning of the subjects, might have influenced the outcome of the study.

Further, we included subjects of two separate studies with differences in the treatment of our control subjects, and so it may be argued that subjects receiving placebo cannot be matched to untreated subjects. Since no differences were observed in hypoxic Vo_2max between the 2007 and 2009 control groups and since both groups presented with lower values than the dexamethasone group, we assume that the fact that only a part of the control group received placebo did not significantly alter the outcome of the study.

It is also true that the two experimental groups were not equal with regard to gender distribution. We do not expect this circumstance to bias our findings. Women can acclimatize and perform at altitude as well as men (West et al., 2007), and the menstrual cycle does not affect performance at high altitude (Beidleman et al., 1999).

Since in our study we included only HAPE-s individuals, we cannot transfer our findings to a general population. However, assuming that subclinical HAPE is a common phenomenon after climbing to Margherita hut within <24 h (Cremona et al., 2002), it is possible that dexamethasone might have the same effect on individuals not susceptible to HAPE, but this will have to be tested separately.

Conclusion

In conclusion, we found that a prophylactic administration of 8 mg dexamethasone b.i.d reduces the hypoxia-related decline in Vo_2max of HAPE-s subjects at 4559-m-high altitude. This is most likely related to an increase in maximal cardiac output and an improved pulmonary O_2 diffusion, both resulting in a higher convectional O_2 transport to the exercising muscles.

Acknowledgments

We gratefully thank the mountaineers that participated in our study and the staff of the Capanna Regina Margherita.

This study was supported by the Zurich Center for Integrative Human Physiology (ZIHP), the Hartmann–Müller Foundation, the Olga-Meyenfisch Foundation, and the Dr. Ettore Crivelli Foundation.

Disclosures

The author's have no conflicts of interest or financial ties to disclose.

References

ACCP. (2003). ATS/ACCP statement on cardiopulmonary exercise testing. Am. J. Respir. Crit. Care Med. 167:211–277.

Arlettaz A., Collomp K., Portier H., Lecoq A.M., Pelle A., and de Ceaurriz J. (2006). Effects of acute prednisolone intake during intense submaximal exercise. Int. J. Sports Med. 27:673–679.

Arlettaz A., Collomp K., Portier H., Lecoq A.M., Rieth N., Le Panse B., and De Ceaurriz J. (2008a). Effects of acute prednisolone administration on exercise endurance and metabolism. Br. J. Sports Med. 42:250–254; discussion 254.

Arlettaz A., Portier H., Lecoq A.M., Labsy Z., de Ceaurriz J., and Collomp K. (2008b). Effects of acute prednisolone intake on substrate utilization during submaximal exercise. Int. J. Sports Med. 29:21–26.

Bartsch P., Mairbaurl H., Maggiorini M., and Swenson E.R. (2005). Physiological aspects of high-altitude pulmonary edema. J. Appl. Physiol. 98:1101–1110.

Bartsch P., Mairbaurl H., Swenson E.R., and Maggiorini M. (2003). High altitude pulmonary oedema. Swiss Med. Weekly. 133:377–384.

Beidleman B.A., Rock P.B., Muza S.R., Fulco C.S., Forte V.A. Jr., and Cymerman A. (1999). Exercise VE and physical performance at altitude are not affected by menstrual cycle phase. J. Appl. Physiol. 86:1519–1526.

Calbet J.A., Boushel R., Radegran G., Sondergaard H., Wagner P.D., and Saltin B. (2003). Determinants of maximal oxygen uptake in severe acute hypoxia. Am. J. Physiol. Regul. Integr. Comp. Physiol. 284:R291–R303.

Calbet J.A., and Lundby C. (2009). Air to muscle O2 delivery during exercise at altitude. High Alt. Med. Biol. 10: 123–134.

Cordova Martinez A. (2006). [Glucocorticoids and sport's performance]. Rev. Clin. Esp. 206:382–384.

Cremona G., Asnaghi R., Baderna P., Brunetto A., Brutsaert T., Cavallaro C., Clark T.M., Cogo A., Donis R., Lanfranchi P., Luks A., Novello N., Panzetta S., Perini L., Putnam M., Spagnolatti L., Wagner H., and Wagner P.D. (2002). Pulmonary extravascular fluid accumulation in recreational climbers: a prospective study. Lancet. 359:303–309.

Duplain H., Vollenweider L., Delabays A., Nicod P., Bartsch P., and Scherrer U. (1999). Augmented sympathetic activation during short-term hypoxia and high-altitude exposure in subjects susceptible to high-altitude pulmonary edema. Circulation. 99:1713–1718.

Ellsworth A.J., Meyer E.F., and Larson E.B. (1991). Acetazolamide or dexamethasone use versus placebo to prevent acute mountain sickness on Mount Rainier. West J. Med. 154:289–293.

Faoro V., Boldingh S., Moreels M., Martinez S., Lamotte M., Unger P., Brimioulle S., Huez S., and Naeije R. (2009). Bosentan decreases pulmonary vascular resistance and improves exercise capacity in acute hypoxia. Chest. 135:1215–1222.

Faoro V., Lamotte M., Deboeck G., Pavelescu A., Huez S., Guenard H., Martinot J.B, and Naeije R. (2007). Effects of sildenafil on exercise capacity in hypoxic normal subjects. High Alt. Med. Biol. 8:155–163.

Fischler M., Maggiorini M., Dorschner L., Debrunner J., Bernheim A., Kiencke S., Mairbaurl H., Bloch K.E., Naeije R., and Brunner-La Rocca H.P. (2009). Dexamethasone but not tadalafil improves exercise capacity in adults prone to high-altitude pulmonary edema. Am. J. Respir. Crit. Care Med. 180:346–352.

Folkesson H.G., Norlin A., Wang Y., Abedinpour P., and Matthay M.A. (2000). Dexamethasone and thyroid hormone pretreatment upregulate alveolar epithelial fluid clearance in adult rats. J. Appl. Physiol. 88:416–424.

Fulco C.S., Rock P.B., and Cymerman A. (1998). Maximal and submaximal exercise performance at altitude. Aviat. Space Environ. Med. 69:793–801.

Ghofrani H.A., Reichenberger F., Kohstall M.G., Mrosek E.H., Seeger T., Olschewski H., Seeger W., and Grimminger F. (2004). Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. Ann. Intern. Med. 141:169–177.

Guney S., Schuler A., Ott A., Hoschele S., Zugel S., Baloglu E., Bartsch P., and Mairbaurl H. (2007). Dexamethasone prevents transport inhibition by hypoxia in rat lung and alveolar epithelial cells by stimulating activity and expression of Na + -K + -ATPase and epithelial Na + channels. Am. J. Physiol. Lung Cell Mol. Physiol. 293:L1332–L1338.

Hopkins S.R., Belzberg A.S., Wiggs B.R., and McKenzie D.C. (1996). Pulmonary transit time and diffusion limitation during heavy exercise in athletes. Respir. Physiol. 103:67–73.

Hultgren H.N. (1996). High-altitude pulmonary edema: current concepts. Annu. Rev. Med. 47:267–284.

Johnson T.S., Rock P.B., Fulco C.S., Trad L.A., Spark R.F., and Maher J.T. (1984). Prevention of acute mountain sickness by dexamethasone. N. Engl. J. Med. 10:683–686.

Lundby C., Boushel R., Robach P., Moller K., Saltin B., and Calbet J.A. (2008). During hypoxic exercise some vasoconstriction is needed to match O2 delivery with O2 demand at the microcirculatory level. J. Physiol. 586:123–130.

Lundby C., Calbet J.A., Sander M., van Hall G., Mazzeo R.S., Stray-Gundersen J., Stager J.M., Chapman R.F., Saltin B., and Levine B.D. (2007). Exercise economy does not change after acclimatization to moderate to very high altitude. Scand. J. Med. Sci. Sports 17:281–291.

Lundby C., Moller P., Kanstrup I.L., and Olsen N.V. (2001). Heart rate response to hypoxic exercise: role of dopamine D2-receptors and effect of oxygen supplementation. Clin. Sci. (Lond). 101:377–383.

Lundby C., Sander M., van Hall G., Saltin B., and Calbet J.A. (2006). Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives. J. Physiol. 573:535–547.

Maggiorini M., Brunner-La Rocca H.P., Peth S., Fischler M., Bohm T., Bernheim A., Kiencke S., Bloch K.E., Dehnert C., Naeije R., Lehmann T., Bartsch P., and Mairbaurl H. (2006). Both tadalafil and dexamethasone may reduce the incidence of high-altitude pulmonary edema: a randomized trial. Ann. Intern. Med. 145:497–506.

Maggiorini M., Muller A., Hofstetter D., Bartsch P., and Oelz O. (1998). Assessment of acute mountain sickness by different score protocols in the Swiss Alps. Aviat. Space Environ. Med. 69:1186–1192.

Marquet P., Lac G., Chassain A.P., Habrioux G., and Galen F.X. (1999). Dexamethasone in resting and exercising men. I. effects on bioenergetics, minerals, and related hormones. J. Appl. Physiol. 87:175–182.

Noda M., Suzuki S., Tsubochi H., Sugita M., Maeda S., Kobayashi S., Kubo H., and Kondo T. (2003). Single dexamethasone injection increases alveolar fluid clearance in adult rats. Crit. Care Med. 31:1183–1189.

Pedersen P.K., Mandoe H., Jensen K., Andersen C., and Madsen K. (1996). Reduced arterial O2 saturation during supine exercise in highly trained cyclists. Acta Physiol. Scand. 158:325–331.

Podolsky A., Eldridge M.W., Richardson R.S., Knight D.R., Johnson E.C., Hopkins S.R., Johnson D.H., Michimata H., Grassi B., Feiner J., Kurdak S.S., Bickler P.E., Severinghaus J.W., and Wagner P.D. (1996). Exercise-induced VA/Q inequality in subjects with prior high-altitude pulmonary edema. J. Appl. Physiol. 81:922–932.

Richalet J.P., Gratadour P., Robach P., Pham I., Dechaux M., Joncquiert-Latarjet A., Mollard P., Brugniaux J., and Cornolo J. (2005). Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. Am. J. Respir. Crit. Care. Med. 171:275–281.

Steinacker J.M., Tobias P., Menold E., Reissnecker S., Hohenhaus E., Liu Y., Lehmann M., Bartsch P., and Swenson E.R. (1998). Lung diffusing capacity and exercise in subjects with previous high altitude pulmonary oedema. Eur. Respir. J. 11:643–650.

Viru A., and Smirnova T. (1982). Independence of physical working capacity from increased glucocorticoid level during short-term exercises. Int. J. Sports Med. 3:80–83.
Vogel J.A., and Harris C.W. (1967). Cardiopulmonary responses of resting man during early exposure to high altitude. J. Appl. Physiol. 22:1124–1128.
Wagner P.D. (2000). New ideas on limitations to VO2max. Exerc. Sport Sci. Rev. 28:10–14.
West J.B., Schoene R.B., and Milledge J.S. (2007). Women at altitude. In: High Altitude Medicine and Physiology, 4th ed. P. Shaw, ed. Hodder Arnold, London; pp. 349–352.

Address correspondence to:
Christoph Siebenmann
Institute of Physiology, Y23 J64
Winterthurerstrasse 190, CH-8057
Zürich, Switzerland

E-mail: christoph.siebenmann@access.uzh.ch

Received November 1, 2010;
accepted in final form January 21, 2011.

Maximal exercise capacity in individuals susceptible to high altitude pulmonary edema at 4559 m.

Christoph Siebenmann[1,2], Konrad E. Bloch[2,3], Carsten Lundby[2], Yvonne Nussbaumer-Ochsner[3], Michèle Schoeb[4], Marco Maggiorini[4]

[1]Institute of Human Movement Sciences and Sport, ETH Zurich, Switzerland
[2]Zurich Center for Integrative Human Physiology (ZIHP), Institute of Physiology, University of Zurich, Switzerland
[3]Pulmonary Division, University Hospital of Zurich, Switzerland
[4]Intensive Care Unit DIM, University Hospital Zurich, Switzerland

Running head: HAPE-susceptibility and exercise

Corresponding author:
Christoph Siebenmann
Institute of Physiology, Y23 J64
Winterthurerstrasse 190
CH-8057 Zürich
Tel: +41 (0)44 635 52 11, E-mail: csiebenm@ethz.ch

To be submitted as a short report within the near future.

Abstract

Background: Subjects susceptible to the development of high altitude pulmonary edema (HAPE-s) experience an exaggerated rise in pulmonary artery pressure when exposed to acute hypoxia. As this may increase right ventricular afterload and/or impair transalveolar O_2-diffusion we hypothesized HAPE-s to experience a more pronounced impairment of maximal O_2 uptake (VO_2max) in hypoxia than normal individuals.

Methods: 14 HAPE-s and 15 normal control subjects (Controls) performed baseline VO_2max-tests on a bicycle-ergometer at sea level. A few weeks later, the tests were repeated after a two-day ascent and an overnight stay at 4559 m altitude. Systolic pulmonary artery pressure (sPpa) was evaluated by trans-thoracic echocardiography at rest and at 20% and 30% of maximal exercise.

Results: Exposure to 4559 m decreased VO_2max by 33% ± 8% in Controls and by 39% ± 6% in HAPE-s ($p=0.052$ for group comparison). SpO_2 during maximal exercise was 72% ± 10% and 74% ± 14%, respectively ($p>0.05$). Exercise elevated sPpa in both groups and at each altitude whereas no difference was observed between groups at sea level. However, at 4559 m, resting sPpa was ~ 12 mmHg higher in HAPE-s than in Controls and this difference persisted at both exercise intensities.

Conclusion: The hypoxia-induced reduction in VO_2max tended to be larger in HAPE-s than in normal individuals. Together with earlier studies that demonstrated an ergogenic effect of pharmacological pulmonary vasodilation in hypoxia this suggests that the pulmonary vasoconstrictive response contributes to the exercise impairment at high altitude.

Introduction

Maximal O_2 uptake (VO_2max) is reduced in acute hypoxia [1, 2] mainly due to two mechanisms [3]: First, a decreased arterial PO_2 (PaO_2) induced by a lower alveolar PO_2 and a resulting pulmonary diffusion limitation [2-4]. Second, a diminished maximal cardiac output due to a decrease in stroke volume and heart rate (HR) [3]. Both mechanisms contribute to a lower O_2 delivery to the exercising muscles and hence a reduction in VO_2max.

This impairment may be even more pronounced in individuals prone to high altitude pulmonary edema (HAPE). HAPE is a noncardiogenic edema, typically occurring after rapid ascent to altitudes > 2500 m [5]. In individuals susceptible to HAPE (HAPE-s), the mechanism of HAPE pathogenesis is an exaggerated vasoconstrictive response to alveolar hypoxia that leads to an excessive and inhomogeneous increase in pulmonary artery resistance [6]. This causes overperfusion of the remaining patent vessels and subsequent injury of capillary walls with leakage of red blood cells and proteins into the airways and alveoli [7].

It is obvious that an overt HAPE impairs exercise tolerance [6, 8]. Nevertheless, the excessive vasoconstrictive response of HAPE-s individuals might also reduce exercise capacity in the absence of a clinically significant pulmonary fluid accumulation. The developing pulmonary hypertension may increase right ventricular afterload, thus attenuating stroke volume [9], and deteriorate ventilation/perfusion matching [10]. Furthermore, even a subclinical extravascular fluid accumulation may elongate the distance for alveolar-capillary O_2-diffusion and thereby amplify pulmonary diffusion limitation [11].

Accordingly, the current study was performed to test the hypothesis that acute exposure to hypoxia induces a larger reduction in VO_2max in HAPE-s than in healthy control subjects (Controls).

Methods

The present results were obtained in the years 2007 and 2009 at the Capanna Regina Margherita (4559 m), a high altitude research facility on Monte Rosa (Italy).
The studies were performed with identical protocols at two occasions to allow inclusion of an appropriate subject number. They were approved by the Ethical Committee of the University of Zurich and conformed to the declaration of Helsinki.

Subjects

The investigation included both, HAPE-s (n=14) and Controls (n=15) as volunteers. All subjects classified as HAPE-s had experienced at least on episode of HAPE in the past as confirmed in an interview by a physician with long experience in high altitude medicine. To prevent bias from former altitude acclimatization, volunteers who spent more than five nights at an altitude higher than 2500 m within the last 30 days before the study were excluded. The anthropometric data of the participants is summarized in table 1.

Table 1: Anthropometric data of the subjects

	Controls	**HAPE-s**
Number of subjects	15	14
Male	11	9
Female	4	5
Age (years)	41 ± 9	45 ± 9
Height (cm)	174 ± 9	174 ± 8
Weight (kg)	73 ± 11	70 ± 7
BMI (kg/m^2)	24 ± 2	23 ± 2

HAPE-s= Subjects susceptible to high altitude pulmonary edema; BMI= Body mass index

Protocol

Baseline examinations were conducted at the University Hospital of Zurich (490 m, referred to as sea level), where subjects performed a VO_2max test at least two hours after the last meal. Two to three weeks later, subjects traveled to Alagna (Italy, 1205 m), from where they were carried by cable car to an altitude of 2900 m. They continued by foot to the Gnifetti hut (3647 m), where they arrived in the late afternoon and spent the night. During the course of the next

morning, they reached the Margherita hut (4559 m) after an additional 4 - 6 hours ascent. After another overnight stay the second VO_2max test was performed in the following morning.

VO2max tests

The VO_2max tests were performed on an electronically braked bicycle ergometer in an upright position (Cyclus 2, RBM Elektronik, Leibzig, Germany). Subjects wore a face mask, covering mouth and nose for breath collection. Amperometric solid-state electrolyte sensors continuously measured O_2- and CO_2-concentration in the expired gas. Results were monitored and saved as breath-by-breath values on a portable computer. The utilized system (ZAN 600 USB, nSpire Health, Louisville, USA) was calibrated before each test. HR was recorded by electrocardiogram (CardioCollect 12, Spacelabs Healthcare, Feucht, Germany) and peripheral arterial O_2 saturation (SpO_2) was measured by pulse oximetry (Masimo SET Radical, Inspiration Medical, Bochum, Germany) on the earlobe.

The trials started at a workload of 50 Watt which was then increased by 10 to 40 Watt per minute following a progressive ramp protocol individually tailored to lead to exhaustion within 8 to 12 min [12]. At high altitude, the increments were thus chosen 30% lower than at sea level. During the last minutes of the test, subjects were vigorously encouraged to continue until exhaustion.

Resting and exercise pulmonary artery pressures

Systolic pulmonary artery pressure (sPpa) was evaluated at rest and during submaximal exercise by trans-thoracic echocardiography, calculated from the pressure gradient across the tricuspid valve using the modified Bernoulli equation and an estimated right atrial pressure of 7mmHg [13]. However, due to technical problems, the measurements of 2007 were lost and thus our results contain only the sPpa values of the 8 HAPE-s and 15 Controls participating in 2009.

Resting sPpa was measured prior to the VO_2max test with the subjects in the supine position. After the VO_2max test, subjects rested for two hours. Subsequently, sPpa was measured with the subjects exercising in the semi-recumbent position on a table ergometer. Measurements were performed at two workloads that were set at 20% and 30% of the maximal workload achieved in the preceding VO_2max test. sPpa was measured after 8 minutes at each workload and exercise was sustained during the procedure.

Data analysis and statistics

Statistical data analysis was conducted using Sigmaplot 11.0. The effect of the exposure to high altitude within each group was evaluated by Wilcoxon matched pairs test. To compare the altitude induced changes between groups, the differences to the baseline results were expressed in percents and compared using student's t-test. sPpA values at sea level and high altitude were compared by paired t-test and changes in sPpa with exercise intensity were tested by ANOVA with Tukey post hoc test. Group differences in sPpa at a given altitude and exercise intensity were tested with student's t-test. Results are given as mean ± SD.

Results

Baseline testing at sea level

Baseline examinations revealed that subjects were healthy, with normal pulmonary functions and free from signs of pulmonary hypertension. All of them were able to perform the exercise tests which revealed no differences between groups (Table 2).

Testing at high altitude

Exposure to altitude was well tolerated in both groups. Despite the ascent to 4559 m within less than 24 hours, no participant had to be treated for acute mountain sickness (AMS) or HAPE and all were able to perform the exercise trials. In both groups, hypoxia induced a significant decrease in VO_2max and maximal workload as well as in HR and SpO_2 during maximal effort (Table 2). The altitude induced reduction in VO_2max was 33 ± 8 % in Controls and 39 ± 6 % in HAPE-s (p=0.052, Figure 1). Otherwise, the altitude-induced changes in all the measured variables were similar between the two groups.

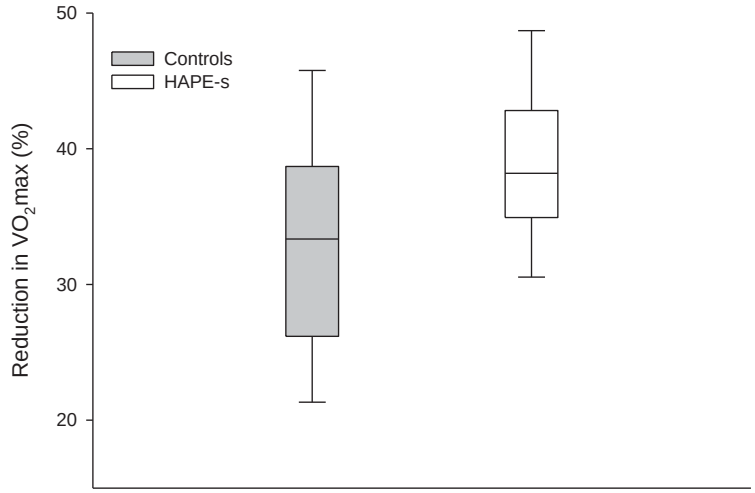

Figure 1: The hypoxia-induced reduction in VO_2max at 4559 m expressed in percentage of sea-level VO_2max (p=0.052 for group comparison). HAPE-s= Subjects susceptible to high altitude pulmonary edema; VO_2max= maximal O_2 uptake.

Pulmonary artery pressures

Figure 3 illustrates sPpa at rest and during 20% and 30% of maximal exercise at both altitudes. At each altitude Exercise increased sPpa in both groups. However, while no differences were present between the groups at sea level, sPpa at rest and both exercise intensities was ~12 mmHg higher in the HAPE-s at high altitude.

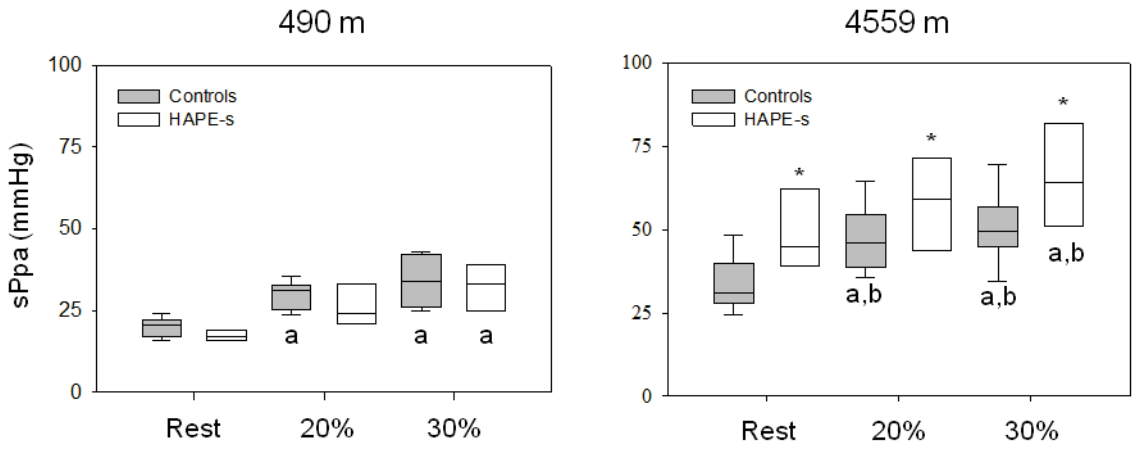

Figure 2: Systolic pulmonary artery pressure (sPpa) at 490 m and 4559 m at rest and 20% and 30% of maximal exercise. *, p<0.05 vs. Controls; a, p<0.05 vs. rest at the same altitude; b, p<0.05 vs. values 490m at the same intensity

Table 2: Cardiopulmonary parameters at maximal exercise capacity

	Zurich, 490m		**Margherita, Day 2, 4559m**		**p-value for altitude effect**	
Group	**Controls**	**HAPE-s**	**Controls**	**HAPE-s**	**Controls**	**HAPE-s**
VO_2max [L/min]	3.7 ±0.9	3.6 ± 0.7	2.4 ± 0.6	2.2 ± 0.4	<0.001	0.002
VCO2 [L/min]	4.2 ± 1	4.2 ± 0.8	2.7 ±0.7	2.4 ± 0.6	<0.001	0.002
Wmax [W]	318 ± 73	279 ± 68	210 ± 64	178 ± 43	<0.001	0.002
HR [1/min]	174 ± 9	179 ±8	162 ± 12	164 ± 12	<0.001	0.003
V_E [L/min]	131 ± 30	131 ± 23	134 ± 39	129 ± 29	0.50	0.75
V_E/VO_2	36.0 ± 4.0	37.1 ± 5.2	55.0 ± 8.6	59.3 ± 8.0	<0.001	0.002
V_E/VCO_2	31.1 ± 3.7	32.0 ± 4.2	49.8 ± 6.0	53.1 ± 7.1	<0.001	0.002
F [1/min]	46 ± 7	46 ±5	50 ± 11	50 ± 10	0.36	0.06
V_T [L]	2.9 ± 0.6	2.9 ± 0.4	2.7 ± 0.65	2.6 ± 0.5	0.01	0.002
F/V_T [1/[L*min]]	16.6 ± 3.6	16.3 ± 3.6	19.6 ± 5.5	20.4 ± 6.4	0.01	0.005
RER	1.16 ± 0.06	1.16 ± 0.04	1.10 ± 0.06	1.12 ± 0.07	0.01	0.08
SpO_2 [%]	95 ± 3	96 ± 3	72 ± 10	74 ± 14	<0.001	0.002

HAPE-s= Subjects susceptible to high altitude pulmonary edema; VO_2max= maximal O_2 uptake; VCO_2= CO_2 output; Wmax= maximal workload; HR= heart rate; V_E= minute ventilation; F= respiratory frequency; V_T= tidal volume; RER= respiratory exchange ratio (VCO_2/VO_2); SpO_2= arterial O_2 Saturation.

Discussion

The major finding of the present study was that the rapid ascent to 4559 m tended (p=0.052) to induce a larger decrease in VO_2max in HAPE-s than in Control subjects which confirms our hypothesis. We further observed sPpa at altitude but not at sea level to be higher in HAPE-s than in Controls both at rest and during moderate exercise.

The cardiovascular response to whole body exercise enhances sPpa even at sea level and in subjects that are not prone to the development of HAPE [14, 15]. As pulmonary vascular resistance was shown not to rise but rather decline during normoxic exercise [16, 17], the increase in sPpa with exercise was attributed to a higher pulmonary blood flow and an enhanced left atrial pressure [14]. In contrast to these findings in a general population, several studies focussed specifically on the exercise response of HAPE-s individuals at sea level [18-23]. In agreement with some [22, 23], but not all of these, the present study did not reveal a higher sPpa in HAPE-s during normoxic exercise. The contrast to some of the earlier studies [18-21] may be related to the exercise intensity since we measured sPpa during moderate exercise to assure appropriate echocardiographic quality. In contrast, previous studies that reported a higher sPpa in HAPE-s individuals [18-21] performed the measurements at more severe intensities. Accordingly, the exercise stimulus in the present experiments may have been too mild to provoke differences between the two groups. This interpretation is supported by the findings of Grunig and co-workers [20] who measured sPpa at different workloads in individuals with and without a history of HAPE and detected an elevated sPpa in HAPE-s only as the workload exceeded 40% of maximal exercise.

In contrast to the results at sea level, sPpa at 4559 m was higher in HAPE-s than in Controls both at rest and during exercise. This is in agreement with earlier observations at the same altitude [13] and in different degrees of hypoxia [6, 21, 23]. The origin of this exaggerated increase in pulmonary artery pressure is still not entirely clarified, but it appears to be multifactorial as several mechanisms have been detected. An important factor seems to be a weak hypoxic ventilatory response which leads to a more marked decrease in alveolar PO_2 and subsequently reinforces the hypoxic pulmonary vasoconstriction [24-26]. Furthermore, an endothelial dysfunction with high plasma levels of endothelin [27, 28] and low levels of NO in hypoxia has been reported [29]. Additional contributors may be a small lung volume with a low cross sectional area of the pulmonary vasculature for blood distribution [19] or an excessive sympathetic activity, leading to increased pulmonary venous pressures [30].

A limiting role of the elevated sPpa in hypoxia has previously been indicated in subjects with [31] or without history of HAPE [9, 32-34] by studies that pharmacologically induced pulmonary vasodilatation by the phosophodiesterase-5-inhibitor Sildenafil [9, 33, 34], the glucocorticoid Dexamethasone [31] or the dual endothelin receptor antagonist bosentan [32]. In these studies the ergogenic actions of the drugs were attributed to a reduced right ventricular afterload [9, 32, 34] and a higher arterial oxygenation due to a lower alveolar fluid accumulation or an optimized ventilation/ perfusion matching [33, 34]. In agreement with these results the elevated sPpa in HAPE-s tended to impair VO_2max in excess of the expected effect of high altitude in the present study. Nevertheless, the natural differences in sPpa between HAPE-s and Control subjects appear to have a minor impact as compared to the changes induced by pharmacological vasodilation. This may be related to a declining relative importance of such natural variations as incremental exercise progressively increased sPpa [14, 15] while the absolute difference between the groups (~ 12 mmHg) remained unchanged (fig. 2). Indeed, the relative difference in sPpa between the groups was 34 % at rest, 26 % at the lower workload and 21 % at the higher workload, indicating a progressive decrease. In contrast, the relative importance of drug-induced reductions in sPpa appears to remain pronounced also at strenuous intensities as indicated in a previous study [9], where even during maximal exercise the difference in sPpa between the Sildenafil- and the Placebo-group was 24 %.

In summary, the present data suggest that the elevated sPpa in HAPE-s might affect VO_2max but rather to a small extent, as long as no clinically overt HAPE develops. Since exercise in hypoxia seems to induce a similar rise in sPpa in individuals susceptible and resistant to HAPE the relative importance of natural differences may decline and be minor during strenuous intensities as compared to pharmacologically induced changes.

Acknowledgment

This study was supported by the Zurich Center for integrative human physiology (ZIHP), the Hartmann-Muller foundation, the Olga-Meyenfisch foundation and the Dr. Ettore Crivelli foundation.

References

1. Calbet, J.A. and C. Lundby, *Air to muscle O2 delivery during exercise at altitude.* High Alt Med Biol, 2009. 10(2): p. 123-34.
2. Wagner, P.D., *New ideas on limitations to VO2max.* Exerc Sport Sci Rev, 2000. 28(1): p. 10-4.
3. Calbet, J.A., et al., *Determinants of maximal oxygen uptake in severe acute hypoxia.* Am J Physiol Regul Integr Comp Physiol, 2003. 284(2): p. R291-303.
4. Lundby, C., et al., *Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives.* J Physiol, 2006. 573(Pt 2): p. 535-47.
5. Bartsch, P., et al., *High altitude pulmonary oedema.* Swiss Med Wkly, 2003. 133(27-28): p. 377-84.
6. Basnyat, B. and D.R. Murdoch, *High-altitude illness.* Lancet, 2003. 361(9373): p. 1967-74.
7. Hultgren, H.N., *High-altitude pulmonary edema: current concepts.* Annu Rev Med, 1996. 47: p. 267-84.
8. Bartsch, P., *High altitude pulmonary edema.* Med Sci Sports Exerc, 1999. 31(1 Suppl): p. S23-7.
9. Ghofrani, H.A., et al., *Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial.* Ann Intern Med, 2004. 141(3): p. 169-77.
10. Podolsky, A., et al., *Exercise-induced VA/Q inequality in subjects with prior high-altitude pulmonary edema.* J Appl Physiol, 1996. 81(2): p. 922-32.
11. Steinacker, J.M., et al., *Lung diffusing capacity and exercise in subjects with previous high altitude pulmonary oedema.* Eur Respir J, 1998. 11(3): p. 643-50.
12. ACCP, A., *ATS/ACCP Statement on cardiopulmonary exercise testing.* Am J Respir Crit Care Med, 2003. 167(2): p. 211-77.
13. Maggiorini, M., et al., *High-altitude pulmonary edema is initially caused by an increase in capillary pressure.* Circulation, 2001. 103(16): p. 2078-83.
14. Bossone, E., et al., *Range of tricuspid regurgitation velocity at rest and during exercise in normal adult men: implications for the diagnosis of pulmonary hypertension.* J Am Coll Cardiol, 1999. 33(6): p. 1662-6.
15. Bevegard, S., A. Holmgren, and B. Jonsson, *Circulatory studies in well trained athletes at rest and during heavy exercise. With special reference to stroke volume and the influence of body position.* Acta Physiol Scand, 1963. 57: p. 26-50.
16. Wagner, P.D., et al., *Pulmonary gas exchange in humans exercising at sea level and simulated altitude.* J Appl Physiol, 1986. 61(1): p. 260-70.
17. Groves, B.M., et al., *Operation Everest II: elevated high-altitude pulmonary resistance unresponsive to oxygen.* J Appl Physiol, 1987. 63(2): p. 521-30.
18. Kawashima, A., et al., *Hemodynamic responses to acute hypoxia, hypobaria, and exercise in subjects susceptible to high-altitude pulmonary edema.* J Appl Physiol, 1989. 67(5): p. 1982-9.

19. Eldridge, M.W., et al., *Pulmonary hemodynamic response to exercise in subjects with prior high-altitude pulmonary edema.* J Appl Physiol, 1996. 81(2): p. 911-21.

20. Grunig, E., et al., *Stress Doppler echocardiography for identification of susceptibility to high altitude pulmonary edema.* J Am Coll Cardiol, 2000. 35(4): p. 980-7.

21. Dehnert, C., et al., *Identification of individuals susceptible to high-altitude pulmonary oedema at low altitude.* Eur Respir J, 2005. 25(3): p. 545-51.

22. Viswanathan, R., et al., *Pulmonary edema of high altitude. II. Clinical, aerohemodynamic, and biochemical studies in a group with history of pulmonary edema of high altitude.* Am Rev Respir Dis, 1969. 100(3): p. 334-41.

23. Hultgren, H.N., R.F. Grover, and L.H. Hartley, *Abnormal circulatory responses to high altitude in subjects with a previous history of high-altitude pulmonary edema.* Circulation, 1971. 44(5): p. 759-70.

24. Hackett, P.H., et al., *Abnormal control of ventilation in high-altitude pulmonary edema.* J Appl Physiol, 1988. 64(3): p. 1268-72.

25. Hohenhaus, E., et al., *Ventilatory and pulmonary vascular response to hypoxia and susceptibility to high altitude pulmonary oedema.* Eur Respir J, 1995. 8(11): p. 1825-33.

26. Matsuzawa, Y., et al., *Blunted hypoxic ventilatory drive in subjects susceptible to high-altitude pulmonary edema.* J Appl Physiol, 1989. 66(3): p. 1152-7.

27. Sartori, C., et al., *Exaggerated endothelin release in high-altitude pulmonary edema.* Circulation, 1999. 99(20): p. 2665-8.

28. Droma, Y., et al., *Endothelin-1 and interleukin-8 in high altitude pulmonary oedema.* Eur Respir J, 1996. 9(9): p. 1947-9.

29. Busch, T., et al., *Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema.* Am J Respir Crit Care Med, 2001. 163(2): p. 368-73.

30. Duplain, H., et al., *Augmented sympathetic activation during short-term hypoxia and high-altitude exposure in subjects susceptible to high-altitude pulmonary edema.* Circulation, 1999. 99(13): p. 1713-8.

31. Fischler, M., et al., *Dexamethasone but not tadalafil improves exercise capacity in adults prone to high-altitude pulmonary edema.* Am J Respir Crit Care Med, 2009. 180(4): p. 346-52.

32. Faoro, V., et al., *Bosentan decreases pulmonary vascular resistance and improves exercise capacity in acute hypoxia.* Chest, 2009. 135(5): p. 1215-22.

33. Faoro, V., et al., *Effects of sildenafil on exercise capacity in hypoxic normal subjects.* High Alt Med Biol, 2007. 8(2): p. 155-63.

34. Richalet, J.P., et al., *Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension.* Am J Respir Crit Care Med, 2005. 171(3): p. 275-81.

Title

Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O_2 uptake

Authors

Christoph Siebenmann[1,2]*, Henrik Sørensen[1,2]*, Robert A. Jacobs[1,3], Thomas Haider[1,3], Peter Rasmussen[1,2], Carsten Lundby[1,2]

*The two authors contributed equally to the manuscript.

Institutions

[1]Zurich Centre for Integrative Human Physiology, [2]Dept. of Physiology, University of Zurich, Switzerland, [3]Vetsuisse, University of Zurich, Switzerland

Running title

Hypocapnia during hypoxic exercise

Address for correspondence

Carsten Lundby, Dept. of Physiol., Winterthurerstrasse 190, CH8057 Zürich, Switzerland.
Carsten.lundby@access.uzh.ch

In press at *Respiratory Physiology and Neurobiology*.

Abstract

With hypoxic exposure ventilation is elevated through the hypoxic ventilatory response. We tested the hypothesis that the resulting hypocapnia reduces maximal exercise capacity by decreasing i) cerebral blood flow and oxygenation and ii) the ventilatory drive.

Eight subjects performed two incremental exercise tests at 3,454m altitude in a blinded manner. In one trial end-tidal P_{CO2} ($P_{ET}CO_2$) was clamped to 40mmHg by CO_2-supplementation. Mean blood flow velocity in the middle cerebral artery ($MCAv_{mean}$) was determined by trans-cranial Doppler sonography and cerebral oxygenation by near infra-red spectroscopy.

Without CO_2-supplementation, $P_{ET}CO_2$ decreased to 30±3mmHg ($P<0.0001$ vs isocapnic trial). Although CO_2-supplementation increased $MCAv_{mean}$ by 17±14% ($P<0.0001$) and attenuated the decrease in cerebral oxygenation (-4.7±0.9% vs -5.4±0.9%; $P=0.002$) this did not affect maximal O_2-uptake. Clamping $P_{ET}CO_2$ increased ventilation during submaximal but not during maximal exercise ($P=0.99$).

We conclude that although hypocapnia promotes a decrease in $MCAv_{mean}$ and cerebral oxygenation, this does not limit maximal O_2-uptake. Furthermore, hypocapnia does not restrict ventilation during maximal hypoxic exercise.

Key words: Altitude, central fatigue, hypoxic ventilatory response, NIRS, respiratory muscle fatigue, ventilatory limitation

Introduction

Hypoxia reduces maximal O_2-uptake ($\dot{V}_{O2}$max) by affecting each step of the O_2 transport cascade (Calbet & Lundby, 2009). At the pulmonary level hypoxia decreases the pressure gradient across the alveolar-capillary membrane limiting diffusive O_2 transport (Wagner *et al.*, 1987). This becomes particularly critical during exercise as the elevated cardiac output shortens pulmonary capillary transit time (Hopkins *et al.*, 1996). The alveolar-arterial P_{O2} difference widens with increasing intensities and the concomitant decrease in arterial O_2 saturation (Sa_{O2}) eventually limits $\dot{V}_{O2}$max (Bebout *et al.*, 1989; Calbet & Lundby, 2009). This is to some extent counteracted by the hypoxic ventilatory response which is activated by stimulation of the peripheral chemoreceptors and elevates pulmonary ventilation ($\dot{V}$E) at rest and during exercise (Klausen *et al.*, 1970; Lahiri *et al.*, 1972). This partially restores alveolar P_{O2} and diffusive O_2 transport (Calbet & Lundby, 2009). Concurrently, the hypoxic ventilatory response reduces arterial P_{CO2} (Pa_{CO2}) (Sutton *et al.*, 1988) which further increases Sa_{O2} by shifting the oxyhemoglobin dissociation curve to the left (Bohr *et al.*, 1904). Despite this positive effect on oxygenation, however, hypoxic ventilatory response induced hypocapnia could negatively affect $\dot{V}_{O2}$max by other mechanisms:

First, hypocapnia may impair $\dot{V}_{O2}$max by a mechanism related to cerebral blood flow (CBF). Fatigue originating from the central nervous system is referred to as central fatigue and may be promoted by an insufficient O_2 delivery to the brain (Amann & Calbet, 2008; Rasmussen *et al.*, 2010). With exercise CBF generally increases up to intensities of 60-80% of maximal capacity where after it plateaus (Querido & Sheel, 2007) or decreases towards or below resting values (Moraine *et al.*, 1993; Hellström *et al.*, 1996). Since CBF is reduced in parallel with Pa_{CO2} as arterial P_{O2} remains constant (Ide *et al.*, 2003), the reduction in CBF during exercise has been attributed to hypocapnia (Poulin *et al.*, 2002; Rasmussen *et al.*, 2006; Bhambhani *et al.*, 2007). Accordingly, blunted CBF combined with low arterial O_2-content in hypoxia causes brain de-oxygenation and may promote central fatigue (Imray *et al.*, 2005; Rasmussen *et al.*, 2006; Subudhi *et al.*, 2009). This hypothesis was recently tested by Subudhi and co-workers (Subudhi *et al.*, 2011) by providing inspiratory CO_2 supplementation during exercise in hypobaric hypoxia corresponding to 4,875 m altitude. This intervention increased CBF and cerebral oxygenation but, contrary to their hypothesis, reduced maximal exercise capacity. We speculated that these intriguing findings might be explained by the severe degree of hypoxia that was

applied. There is indeed evidence that, for submaximal exercise, cerebral de-oxygenation only becomes a limiting factor when Sa_{O2} falls below 82% (Amann & Calbet, 2008). However, maximal exercise capacity at altitudes > 4,000 m is crucially reduced by a decrease in maximal cardiac output (Calbet *et al.*, 2003) and peripheral limitations (Lundby *et al.*, 2008; Robach *et al.*, 2008) which may together have overruled a potential benefit from an increased cerebral oxygenation. We therefore aimed to investigate whether the recent findings of Subudhi and colleagues (Subudhi *et al.*, 2011) could be confirmed at an altitude that is < 4000 m, yet still in a range where effects of cerebral de-oxygenation on exercise performance have been observed previously (Goodall *et al.*, 2012).

A second mechanism by which hypocapnia may impair $\dot{V}_{O2}max$ in hypoxia is attenuation of the ventilatory drive (Dempsey, 1976) as this effect could amplify the hypoxemia during maximal exercise. At sea level, inspiratory CO_2 supplementation elevates maximal exercise $\dot{V}E$ in young subjects (Babb, 1997), suggesting an inhibitory effect of hypocapnia rather than a mechanical limitation. Since the mechanical constraints associated with ventilation may be reduced in hypobaric hypoxia (Mognoni *et al.*, 1982) while at the same time hypocapnia is more pronounced (Sutton *et al.*, 1988), we speculated the blunting effect of hypocapnia on $\dot{V}E$ to persist or become more pronounced than at sea level.

In summary, the aim of the present study was to investigate the impact of hypocapnia on exercise at 3,454 m altitude. We hypothesized that clamping $P_{ET_{CO2}}$ to 40 mmHg would increase i) CBF and cerebral oxygenation and ii) $\dot{V}E$ during exercise. We further expected one of these mechanisms or a combination hereof to increase $\dot{V}_{O2}max$.

Methods

Subjects

Eight healthy subjects were recruited (5 males: 28±1 y, 77±10 kg, 182±5 cm, and 3 females: 27±1 yrs, 47±5 kg, 163±5 cm). The study was conducted at the Jungfraujoch research station (3,454 m) in the Swiss Alps. It was approved by the local ethical committee (EK-2011-N-21) in accordance with the declaration of Helsinki. Prior to the start of the experiments, informed oral and written consents were obtained.

Protocol

Subjects were transported by train to the Jungfraujoch on the evening prior to the experiments and spent the night at the research station. The following day they performed two incremental exercise tests to exhaustion on an electronically braked bicycle ergometer (Monark 839E, Varberg, Sweden) breathing either ambient air (hypocapnic trial) or CO_2-enriched air (isocapnic trial). In the latter the inspired CO_2 fraction was continuously adjusted (Altitrainer, SMTEC, Nyon, Switzerland) to clamp $P_{ET}CO_2$ at 40 mmHg. The order of the trials were randomized and subjects, but not the investigators, were blinded. The trials were separated by at least four hours where subjects had a snack and beverage. Both exercise trials followed the same protocol starting with a warm-up period of 10 min at 100 W (males) or at 80 W (females). Thereafter the workload was increased every minute by 25 or 20 W, respectively, until exhaustion. Verbal encouragement was given in the end of all trials. Maximal workloads completed in the exercise tests were calculated as $W_{max} = W_{compl} + W_{increm} \times (t/60)$ with W_{compl} being the last completed workload, W_{increm} the workload increment per exercise step and t the number of seconds in the not completed workload.

Cerebral blood flow and cerebral oxygenation

The mean blood flow velocity in the middle cerebral artery (MCA v_{mean}) was determined as an estimate of CBF by insonating through the temporal window by trans-cranial Doppler sonography (2MHz probe, Multi-Box, DWL, Singen, Germany; ST3 Digital, Spencer Technologies, Northbrough, USA).

This approach is based on the assumption that the diameter of the MCA does not change during the measurement which we did not confirm experimentally. However, since an influence of Pa_{CO_2} on the MCA diameter has previously been excluded (Serrador *et al.*, 2000) we were confident that the changes observed in MCA v_{mean} reflected those in CBF. After the lowest signal to noise ratio was obtained the insonation probe was fastened to a headband and secured with adhesive sonography gel.

To assess cerebral oxygenation we used near infra-red spectroscopy (NIRS, NIRO-200, Hamamatsu, Japan), which exploits spatial resolution to attenuate the influence from superficial tissues. Although this technique is still affected by skin blood flow and cerebrospinal fluid (Yoshitani *et al.*, 2007; Sørensen *et al.*, 2012) it may detect changes in cerebral oxygenation during hypoxemia (Sørensen *et al.*, 2012). We did not expect any differences between hemispheres, therefore the sensor was applied ipsilaterally to the Doppler probe. The sensor was placed high on the forehead to avoid influence from the frontal and saggital sinus.

Ventilatory variables

Subjects wore a mask covering nose and mouth for complete breath collection (Hans Rudolf, Kansas City, USA). The ventilatory exercise response was measured breath-by-breath by a spirometer (Cosmed Quark CPET, Rome, Italy). After the test, the highest average value for $\dot{V}_{O_2}$ calculated over 30 breaths was adopted as $\dot{V}_{O_2}max$. Pa_{CO_2} was calculated from tidal volume and end-tidal P_{CO_2} according to Jones et al. (Jones *et al.*, 1979). A pulse oximeter (LifeSense, Nonin Medical Inc., Plymouth, USA) was applied to the subjects' fingertip to determine Sa_{O_2} and heart rate (f_H) was measured by a monitor belt (Polar Electro, Kempele, Finland).

Statistical analysis

Data were analyzed by two-way ANOVA on repeated measurements and given as mean with SEM. The statistical significance level was set to $P < 0.05$. The analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, USA).

Results

$P_{ET_{CO_2}}$ and Pa_{CO_2}

In the isocapnic trial $P_{ET_{CO_2}}$ was clamped to 40±1 mmHg throughout the whole test (Fig. 1a). In the hypocapnic trial $P_{ET_{CO_2}}$ decreased from 35±1 mmHg (P<0.0001 vs isocapnic trial) to 30±3 during maximal exercise (P<0.0001). Similarly, Pa_{CO_2} decreased in parallel with $P_{ET_{CO_2}}$ in the hypocapnic trial but remained close to 40 mmHg in the isocapnic trial (P<0.0001).

Exercise capacity

Clamping of $P_{ET_{CO_2}}$ had no effect on $\dot{V}_{O_2}max$ which was 3.3±1.0 $l.min^{-1}$ in the hypocapnic and 3.2±1.0 $l.min^{-1}$ in the normocapnic trial (P=0.57). The $\dot{V}_{O_2}$ response at different workloads is illustrated in Fig. 1b. The maximal f_H was 182±10 in the hypocapnic and 180±8 in the isocapnic trial, respectively (P=0.30). The corresponding maximally attained workloads were 243±68 W and 229±62 W (P=0.04).

Cerebral blood flow and cerebral oxygenation

In the hypocapnic trial MCA v_{mean} increased with exercise (Fig. 2a) and reached a plateau approximately 20% above resting values (P<0.0001). In the isocapnic trial MCA v_{mean} followed the same pattern but reached a higher plateau approximately 35% above resting values (P<0.0001). Furthermore, the exercise induced reduction in cerebral oxygenation was less pronounced in the isocapnic trial versus the hypocapnic trial (-5.4±0.9% vs. -4.7±0.9%; P=0.002, Fig. 2b). Individual data analysis revealed no correlation between changes in cerebral oxygenation and exercise capacity.

Ventilatory variables

Clamping $P_{ET_{CO_2}}$ tended to increase $\dot{V}_E$ during submaximal exercise but this effect was abolished with increasing intensity (Fig. 1c). Similarly, $P_{ET_{O_2}}$ was elevated during the warm-up workload but

decreased to the levels observed in the hypocapnic trial with increasing exercise intensity (Fig. 1d). Other ventilatory variables during maximal exercise are summarized in Table 1.

Pulse oximetry failed to provide a stable signal throughout the exercise trials. Accordingly, the Sa_{O_2} response could not be analyzed.

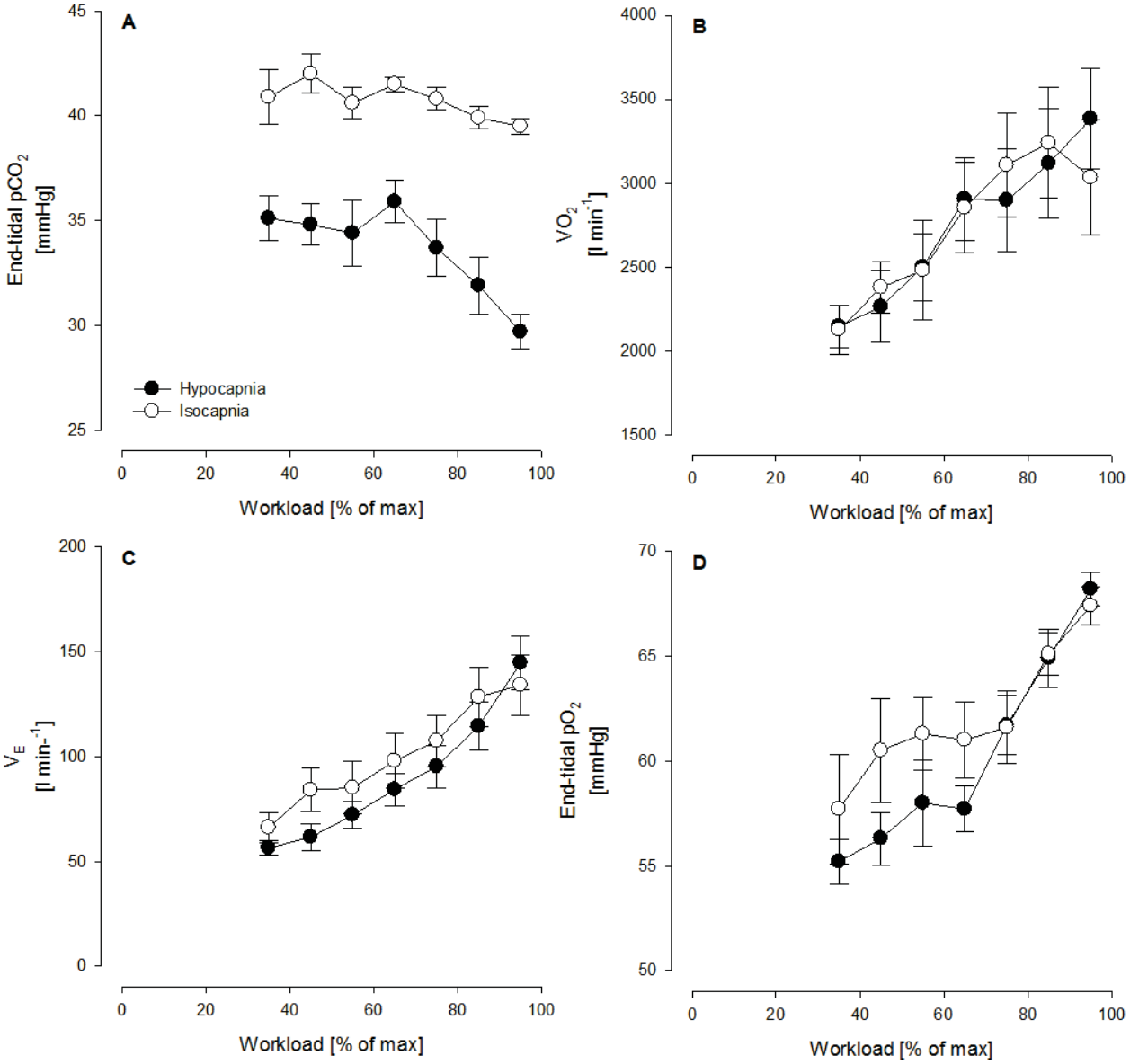

Figure 1: Ventilatory variables during incremental exercise at 3,454m with (isocapnia) or without (hypocapnia) CO_2 added to the inspirate to maintain end-tidal PCO_2 at ~40mmHg. Values are mean ± SEM for N=8. $\dot{V}O_2$, pulmonary O_2 uptake; $\dot{V}E$, pulmonary ventilation.

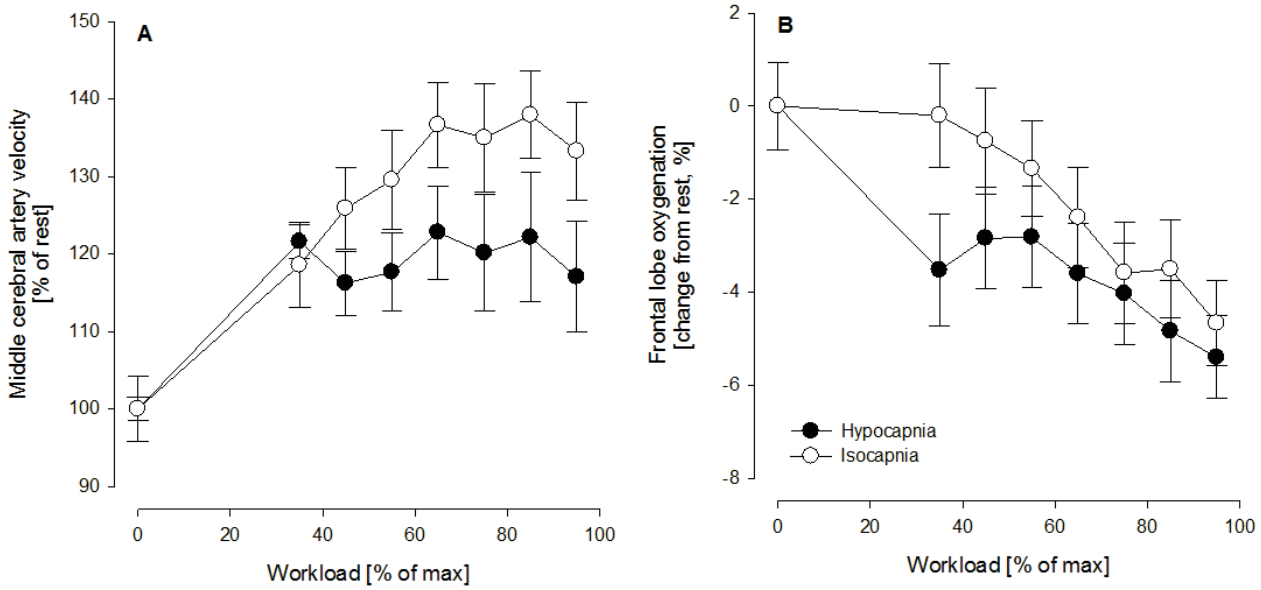

Figure 2: Middle cerebral artery mean velocity (**A**) and frontal lobe oxygenation (**B**) during incremental exercise at 3,454m with (isocapnia) or without (hypocapnia) CO_2 added to the inspirate to maintain end-tidal PCO_2 at ~40mmHg. Values are mean ± SEM for N=8.

Table 1: Ventilatory variables during maximal exercise

Hypocapnia	Respiration frequency [breath.min^{-1}]	50.8 ± 8.7
	Tidal volume [l]	2.8 ± 0.9
	Ventilation [l.min-1]	140 ± 42
	Pulmonary $\dot{V}_{O2}$ [l.min^{-1}]	3.3 ± 1.0
	Pulmonary $\dot{V}_{CO2}$ [l.min^{-1}]	3.8 ± 1.1
	End-tidal P_{CO2} [mmHg]	29.6 ± 3.1
	Calculated arterial P_{CO2} [mmHg]	31.5 ± 2.7
Isocapnia	Respiration frequency [breath.min^{-1}]	47.7 ± 8.0
	Tidal volume [l]	3.0 ± 0.9
	Ventilation [l.min-1]	141 ± 46
	Pulmonary $\dot{V}_{O2}$ [l.min^{-1}]	3.1 ± 1.1
	Pulmonary $\dot{V}_{CO2}$ [l.min^{-1}]	3.5 ± 1.2
	End-tidal P_{CO2} [mmHg]	39.3 ± 0.8
	Calculated arterial P_{CO2} [mmHg]	40.3 ± 0.8

Hypocapnia, control exercise; isocapnia, CO_2 added to the inspirate to maintain end-tidal P_{CO2} at ~40mmHg. Values are mean ± SD for N=8.

Discussion

We tested the hypothesis that hypocapnia during exercise at an altitude of 3,454 m promotes a decrease in cerebral oxygenation and blunts $\dot{V}_E$. We further speculated that these responses could contribute to the reduction in $\dot{V}_{O_2}max$ in hypoxia. In agreement with our hypothesis, clamping $P_{ET}CO_2$ at 40 mmHg increased MCA v_{mean}, as an estimate of CBF, and diminished the reduction in cerebral oxygenation. This, however, did not affect $\dot{V}_{O_2}max$ and argues against cerebral deoxygenation as an important limiting factor for incremental exercise in hypoxia. Furthermore, although the intervention tended to increase $\dot{V}_E$ during submaximal workloads, this effect disappeared with increasing intensities, suggesting that other factors constrain $\dot{V}_E$ during maximal exercise in hypoxia.

Cerebral blood flow and cerebral oxygenation

Central fatigue is defined as a decrease in neural motor drive despite a maintained or even increasing demand for muscle force generation (Gandevia *et al.*, 1995). One proposed mechanism is that accumulation of fatigue-related metabolites in the contracting skeletal muscles increases the discharge rate in group III/IV afferents which, in turn, causes reflex inhibition of the α-motoneuron firing rate and thus limits further muscle contraction (Gandevia, 2001; Amann *et al.*, 2007). Hypoxia promotes this process not only by accelerating the accumulation of fatigue-related muscle metabolites (Linnarsson *et al.*, 1974) but also by increasing the resting firing rate of the III/IV afferents (Hill *et al.*, 1992). Apart from this indirect pathway, hypoxia may also directly affect the central nervous system by depriving cerebral oxygenation (Amann & Calbet, 2008). Indirect support for this mechanism in humans is found in studies where subjects were switched to breathing a hyperoxic gas mixture immediately before exhausting in a hypoxic exercise test (Calbet *et al.*, 2003; Amann *et al.*, 2007; Subudhi *et al.*, 2008). This restored cerebral oxygenation within seconds and enabled the subjects to continue exercising for several minutes. However, this approach is limited by the obvious global effect of hyperoxic breathing that restores oxygenation in all tissues. Accordingly, the isolated effect of the cerebral re-oxygenation could not be determined. To overcome this limitation Subudhi and co-workers manipulated cerebral oxygenation independent of Sa_{O_2} by increasing MCA v_{mean} through inspiratory CO_2 supplementation (Subudhi *et al.*, 2011). This intervention successfully elevated cerebral oxygenation but, contrary to their hypothesis, decreased maximal exercise capacity. We

speculated that the severe degree of hypoxia applied by Subudhi and colleagues, corresponding to 4,875 m, (Subudhi *et al.*, 2011) induced cardiac (Calbet *et al.*, 2003) and peripheral (Lundby *et al.*, 2008; Robach *et al.*, 2008) limitations to exercise that may have overruled a potential positive effect of the elevated cerebral oxygenation. Although there is evidence that, during submaximal exercise, cerebral hypoxia becomes a limiting factor only in severe hypoxia (Amann & Calbet, 2008), more recent studies observed an effect at an altitude that was similar to the one in the present experiments (Goodall *et al.*, 2012) or even at sea level (Rasmussen *et al.*, 2010). It should also be considered that incremental exercise, as applied in the present study, induces a progressive decrease in Sa_{O_2} (Hughes *et al.*, 1968; Calbet *et al.*, 2003) that eventually results in more pronounced hypoxemia than during submaximal exercise at the same altitude. Accordingly, we speculated that at the more moderate altitude of 3,454 m, where peripheral limitations are less pronounced and cardiac output is not curtailed, an increase in CBF and cerebral oxygenation may benefit maximal exercise capacity. Similar to the previous study (Subudhi *et al.*, 2011), where MCA v_{mean} tended ($P<0.10$) to be higher with CO_2 clamping, MCA v_{mean} in the present study increased starting from approximately 35% of maximal exercise. Despite this, and similar to the earlier observations (Subudhi *et al.*, 2011), the associated increase in cerebral oxygenation did not lead to an increase in $\dot{V}_{O_2}max$. These findings indicate that the diminished cerebral oxygenation during hypoxic exercise is not an important limiting factor for $\dot{V}_{O_2}max$. However, it has to be considered that inspiratory CO_2 supplementation may have induced restrictions to exercise that overruled a potential benefit of the elevated cerebral oxygenation. The intervention may have prevented a ventilatory compensation of the metabolic acidosis associated with heavy exercise (Wasserman, 1986). This could have impaired $\dot{V}_{O_2}max$ either by a direct influence on the exercising muscles (Sahlin, 1986) or indirectly by shifting the oxyhemoglobin curve to the right and thus decreasing Sa_{O_2} (Lundby *et al.*, 2006). This is supported by the lower workloads reached in the isocapnic trial. Future studies could prevent this effect by maintaining arterial pH with bicarbonate supplementation.

Ventilation

The major determinant of $\dot{V}_{O_2}max$ is convective O_2 delivery to the exercising muscles (Wagner, 1996). In normally trained individuals the capacity of the pulmonary system is generally sufficient to prevent major O_2 de-saturations (Bassett & Howley, 2000) and accordingly $\dot{V}_{O_2}max$ is primarily limited by the capacity to increase cardiac output (Ekblom & Hermansen, 1968; Coyle *et al.*, 1984). Hypoxia, in contrast, reduces alveolar P_{O_2} which attenuates the alveolar-capillary pressure gradient and thereby attenuates trans-alveolar O_2 diffusion. Consequently, Sa_{O_2} decreases gradually (Hughes *et al.*, 1968; Calbet *et al.*, 2003) secondary to an elevated cardiac output during exercise which shortens pulmonary capillary transit times (Hopkins *et al.*, 1996). Since maximal cardiac output is unaffected in moderate hypoxia as compared to in normoxia (Stenberg *et al.*, 1966; Hartley *et al.*, 1973) each decline in Sa_{O_2} reduces convective O_2 transport and thus $\dot{V}_{O_2}max$ (Calbet *et al.*, 2003). Compensatory hyperpnoea counteracts this process by enhancing alveolar P_{O_2} and partially restoring the driving pressure for diffusive O_2 transport. Nonetheless, despite the hypoxic ventilatory response, $\dot{V}E$ remains insufficient to abolish the progressive hypoxemia during incremental exercise in hypoxia. This raises the question as to what prevents further elevations in $\dot{V}E$ despite an increasing hypoxic stimulation of the peripheral chemoreceptors. We speculated the hypocapnia that develops secondary to the hypoxic ventilatory response (Sutton *et al.*, 1988) to be a potential candidate. Hypocapnia is a potent antagonist to the ventilatory drive and has been reported to confine the hypoxic ventilatory response in resting humans (Huang *et al.*, 1984; Steinback & Poulin, 2007). However, suppression of hypocapnia by inspiratory CO_2 administration only tended to increase $\dot{V}E$ at submaximal workloads and was without effect during maximal exercise. This is in agreement with earlier observations in young amateur cyclists at a higher simulated altitude (Subudhi *et al.*, 2011) and indicates that maximal $\dot{V}E$ in hypoxia is confined by other mechanism. An obvious candidate is the mechanical restriction associated with higher air flows (Guenette & Sheel, 2007). By applying inspiratory CO_2 or Helium supplementation Babb (Babb, 1997) has previously demonstrated in the elderly, that mechanical restrictions may limit maximal exercise $\dot{V}E$ at sea level. In that study unloading of the airways by Helium increased maximal but not submaximal exercise V_E. In contrast and similar to the present results CO_2 supplementation only stimulated $\dot{V}E$ during submaximal workloads and had no effect during maximal exercise. A similar effect of airway unloading was recently observed in mild hypobaric hypoxia corresponding to 2,500 m altitude (Ogawa *et al.*, 2010). Nevertheless, we hypothesized the effect of hypocapnia on $\dot{V}E$

to dominate during exercise at higher altitude where the work of breathing may be reduced (Mognoni *et al.*, 1982) while hypocapnia on the other hand is more pronounced. However, our results do not support this hypothesis and suggest that, at 3,454 m altitude, mechanical flow limitations may persist and prevent CO_2 supplementation from increasing V_E. This is further supported by the observation that $\dot{V}E$ during maximal exercise at altitude may match maximal voluntary ventilation (Forte *et al.*, 1997). It has to be considered that the prediction of the highest attainable $\dot{V}E$ during exercise by a maximal voluntary ventilation manoeuvre is contentious since the voluntary breathing pattern differs from reflex ventilation (Jensen *et al.*, 1980) and the test outcome is depending on the duration of the manoeuvre (Kift & Williams, 2008). Nevertheless, these confounding variables lead to an over- rather than underestimate of maximal exercise $\dot{V}E$ (Kift & Williams, 2008) and thus the absence of a ventilatory reserve at altitude (Forte *et al.*, 1997) clearly suggests ventilatory limitation.

Another mechanism that may have prevented the CO_2 supplementation from increasing maximal $\dot{V}E$ is respiratory muscle fatigue. Previous work has demonstrated that the diaphragm and abdominal muscles are susceptible to fatigue during sustained high intensity exercise (Romer & Polkey, 2008). Generally, this is only observed when heavy exercise (>85% of $\dot{V}_{O_2}max$) is sustained for more than 8-10 minutes (Johnson *et al.*, 1996) and not during incremental exercise tests (Romer *et al.*, 2007). However, it should be considered that the development of respiratory muscle fatigue may be accelerated in hypoxia (Verges *et al.*, 2010) and we can thus not exclude a limiting role hereof in the present study although experimental support is at present lacking.

In summary, the present results indicate that hypocapnia does not limit $\dot{V}E$ during maximal exercise in hypoxia and indicate a role of other mechanisms like mechanical limitation or potentially respiratory muscle fatigue.

Limitations

In the present study we tested whether changes in cerebral oxygenation affect $\dot{V}_{O_2}max$ at altitude. Due to the design of our experiments, which included inspiratory CO_2 supplementation, we could not confirm the achievement of a true $\dot{V}_{O_2}max$ by standard criteria (ATS/ACCP, 2003). A clear plateau in the $\dot{V}O_2$ response was reached in both trials by six subjects but not by the remaining two subjects. Nevertheless, since the maximal f_H was similar between the two trials we are confident that the present results cannot be explained by insufficient subject effort during the isocapnic trial. A further limitation is that we assessed differences in CBF by measurements of MCA v_{mean}. This approach is based on the assumption that the diameter of the insonated vessel was similar between trials. As indicated, a recent report excludes the influence of Pa_{CO_2}on the arterial vessel diameter (Serrador *et al.*, 2000). Furthermore, an increase in middle cerebral artery diameter in the isocapnic trial would have led to an under- rather than overestimation of CBF and thus our conclusions that the intervention increased CBF remains valid. It also has to be considered that the assessment of cerebral oxygenation by NIRS may be affected by changes in skin blood flow (Sørensen *et al.*, 2012). We argue, however, that skin blood flow was likely similar between the two trials and thus cannot explain the difference in NIRS derived cerebral oxygenation. Finally, although our observations do not support the hypothesis that a decrease in cerebral oxygenation affects maximal exercise capacity in hypoxia we cannot rule out that this may have an effect on submaximal exercise performance. Future studies could investigate whether inspiratory CO_2 supplementation increases time trial performance at altitude.

Conclusion

Although clamping of $P_{ET_{CO_2}}$ increased MCA v_{mean}, as an estimate for CBF and augmented cerebral oxygenation this did not increase $\dot{V}_{O_2}max$. Our results suggest that cerebral oxygenation is not a limiting factor for incremental exercise in hypoxia although it remains unknown whether acidosis due to CO_2-supplementation overrode a potential effect. Our results further indicate that hypocapnia does not curtail $\dot{V}_E$ during maximal exercise at 3,454m altitude.

Acknowledgement

The authors kindly thank the staff of the Jungfraujoch Research Station for their logistical and practical support. This study was funded by the Zurich Center for Intergrative Human Physiology (ZHIP). The author HS was supported by a grant from Direktør Ib Henriksen Fonden, Oticon Fonden and Prosektor Axel Søeborg Ohlsen og ægtefælles Mindelegat.

References

Amann M & Calbet JA. (2008). Convective oxygen transport and fatigue. *J Appl Physiol* 104, 861-870.

Amann M, Romer LM, Subudhi AW, Pegelow DF & Dempsey JA. (2007). Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *The Journal of physiology* 581, 389-403.

ATS/ACCP. (2003). ATS/ACCP Statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 167, 211-277.

Babb TG. (1997). Ventilatory response to exercise in subjects breathing CO2 or HeO2. *J Appl Physiol* 82, 746-754.

Bassett DR, Jr. & Howley ET. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32, 70-84.

Bebout DE, Story D, Roca J, Hogan MC, Poole DC, Gonzalez-Camarena R, Ueno O, Haab P & Wagner PD. (1989). Effects of altitude acclimatization on pulmonary gas exchange during exercise. *Journal of applied physiology* 67, 2286-2295.

Bhambhani Y, Malik R & Mookerjee S. (2007). Cerebral oxygenation declines at exercise intensities above the respiratory compensation threshold. *Respiratory physiology & neurobiology* 156, 196-202.

Bohr C, Hasselbalch K & Krogh A. (1904). About a new biological relation of high importance that the blood carbonic acid tension exercises on its oxygen binding. *Skand Arch Physiol* 16, 402-412.

Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD & Saltin B. (2003). Determinants of maximal oxygen uptake in severe acute hypoxia. *Am J Physiol Regul Integr Comp Physiol* 284, R291-303.

Calbet JA & Lundby C. (2009). Air to muscle O2 delivery during exercise at altitude. *High Alt Med Biol* 10, 123-134.

Coyle EF, Martin WH, 3rd, Sinacore DR, Joyner MJ, Hagberg JM & Holloszy JO. (1984). Time course of loss of adaptations after stopping prolonged intense endurance training. *J Appl Physiol* 57, 1857-1864.

Dempsey JA. (1976). CO2 response: stimulus definition and limitations. *Chest* 70, 114-118.

Ekblom B & Hermansen L. (1968). Cardiac output in athletes. *J Appl Physiol* 25, 619-625.

Forte VA, Jr., Leith DE, Muza SR, Fulco CS & Cymerman A. (1997). Ventilatory capacities at sea level and high altitude. *Aviat Space Environ Med* 68, 488-493.

Gandevia SC. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81, 1725-1789.

Gandevia SC, Allen GM & McKenzie DK. (1995). Central fatigue. Critical issues, quantification and practical implications. *Adv Exp Med Biol* 384, 281-294.

Goodall S, Gonzalez-Alonso J, Ali L, Ross EZ & Romer LM. (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol*.

Guenette JA & Sheel AW. (2007). Physiological consequences of a high work of breathing during heavy exercise in humans. *J Sci Med Sport* 10, 341-350.

Hartley LH, Vogel JA & Landowne M. (1973). Central, femoral, and brachial circulation during exercise in hypoxia. *J Appl Physiol* 34, 87-90.

Hellström G, Fischer-Colbrie W, Wahlgren NG & Jögestrand T. (1996). Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise. *J Appl Physiol* 81, 413-418.

Hill JM, Pickar JG, Parrish MD & Kaufman MP. (1992). Effects of hypoxia on the discharge of group III and IV muscle afferents in cats. *J Appl Physiol* 73, 2524-2529.

Hopkins SR, Belzberg AS, Wiggs BR & McKenzie DC. (1996). Pulmonary transit time and diffusion limitation during heavy exercise in athletes. *Respir Physiol* 103, 67-73.

Huang SY, Alexander JK, Grover RF, Maher JT, McCullough RE, McCullough RG, Moore LG, Sampson JB, Weil JV & Reeves JT. (1984). Hypocapnia and sustained hypoxia blunt ventilation on arrival at high altitude. *J Appl Physiol* 56, 602-606.

Hughes RL, Clode M, Edwards RH, Goodwin TJ & Jones NL. (1968). Effect of inspired O2 on cardiopulmonary and metabolic responses to exercise in man. *J Appl Physiol* 24, 336-347.

Ide K, Eliasziw M & Poulin MJ. (2003). The relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* 95, 129-137.

Imray CHE, Myers SD, Pattinson KTS, Bradwell AR, Chan CW, Harris S, Collins P, Wright AD & Society tBMRE. (2005). Effect of exercise on cerebral perfusion in humans at high altitude. *Journal of Applied Physiology* 99, 699-706.

Jensen JI, Lyager S & Pedersen OF. (1980). The relationship between maximal ventilation, breathing pattern and mechanical limitation of ventilation. *J Physiol* 309, 521-532.

Johnson BD, Aaron EA, Babcock MA & Dempsey JA. (1996). Respiratory muscle fatigue during exercise: implications for performance. *Med Sci Sports Exerc* 28, 1129-1137.

Jones NL, Robertson DG & Kane JW. (1979). Difference between end-tidal and arterial PCO2 in exercise. *J Appl Physiol* 47, 954-960.

Kift J & Williams E. (2008). Ventilatory capacity and its utilisation during exercise. *Lung* 186, 345-350.

Klausen K, Dill DB & Horvath SM. (1970). Exercise at ambient and high oxygen pressure at high altitude and at sea level. *J Appl Physiol* 29, 456-463.

Lahiri S, Milledge JS & Sorensen SC. (1972). Ventilation in man during exercise at high altitude. *J Appl Physiol* 32, 766-769.

Linnarsson D, Karlsson J, Fagraeus L & Saltin B. (1974). Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* 36, 399-402.

Lundby C, Boushel R, Robach P, Moller K, Saltin B & Calbet JA. (2008). During hypoxic exercise some vasoconstriction is needed to match O2 delivery with O2 demand at the microcirculatory level. *The Journal of physiology* 586, 123-130.

Lundby C, Sander M, van Hall G, Saltin B & Calbet JA. (2006). Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives. *J Physiol* 573, 535-547.

Mognoni P, Saibene F & Veicsteinas A. (1982). Ventilatory work during exercise at high altitude. *Int J Sports Med* 3, 33-36.

Moraine JJ, Lamotte M, Berre J, Niset G, Leduc A & Naeije R. (1993). Relationship of middle cerebral artery blood flow velocity to intensity during dynamic exercise in normal subjects. *Eur J Appl Physiol Occup Physiol* 67, 35-38.

Ogawa T, Calbet JA, Honda Y, Fujii N & Nishiyasu T. (2010). The effects of breathing a helium-oxygen gas mixture on maximal pulmonary ventilation and maximal oxygen consumption during exercise in acute moderate hypobaric hypoxia. *Eur J Appl Physiol* 110, 853-861.

Poulin MJ, Fatemian M, Tansley JG, O'Connor DF & Robbins PA. (2002). Changes in cerebral blood flow during and after 48 h of both isocapnic and poikilocapnic hypoxia in humans. *Experimental physiology* 87, 633-642.

Querido JS & Sheel AW. (2007). Regulation of cerebral blood flow during exercise. *Sports Med* 37, 765-782.

Rasmussen P, Nielsen J, Overgaard M, Krogh-Madsen R, Gjedde A, Secher NH & Petersen NC. (2010). Reduced muscle activation during exercise related to brain oxygenation and metabolism in humans. *J Physiol* 588, 1985-1995.

Rasmussen P, Stie H, Nielsen B & Nybo L. (2006). Enhanced cerebral CO_2 reactivity during strenuous exercise in man. *Eur J Appl Physiol* 96, 299-304.

Robach P, Calbet JAL, Thomsen JJ, Boushel R, Mollard P, Rasmussen P & Lundby C. (2008). The Ergogenic Effect of Recombinant Human Erythropoietin on V̇O2max Depends on the Severity of Arterial Hypoxemia. *PLoS ONE* 3, e2996.

Romer LM, Miller JD, Haverkamp HC, Pegelow DF & Dempsey JA. (2007). Inspiratory muscles do not limit maximal incremental exercise performance in healthy subjects. *Respir Physiol Neurobiol* 156, 353-361.

Romer LM & Polkey MI. (2008). Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol* 104, 879-888.

Sahlin K. (1986). Muscle fatigue and lactic acid accumulation. *Acta Physiol Scand Suppl* 556, 83-91.

Serrador JM, Picot PA, Rutt BK, Shoemaker JK & Bondar RL. (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke* 31, 1672-1678.

Sørensen H, Secher NH, Siebenmann C, Nielsen HB, Kohl-Bareis M, Lundby C & Rasmussen P. (2012). Cutaneous vasoconstriction affects near-infrared spectroscopy determined cerebral oxygen saturation during administration of norepinephrine. *Anesthesiology* In Press.

Steinback CD & Poulin MJ. (2007). Ventilatory responses to isocapnic and poikilocapnic hypoxia in humans. *Respir Physiol Neurobiol* 155, 104-113.

Stenberg J, Ekblom B & Messin R. (1966). Hemodynamic response to work at simulated altitude, 4,000 m. *J Appl Physiol* 21, 1589-1594.

Subudhi AW, Lorenz MC, Fulco CS & Roach RC. (2008). Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. *Am J Physiol Heart Circ Physiol* 294, H164-171.

Subudhi AW, Miramon BR, Granger ME & Roach RC. (2009). Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *Journal of Applied Physiology* 106, 1153-1158.

Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B & Roach RC. (2011). Does cerebral oxygen delivery limit incremental exercise performance? *J Appl Physiol* 111, 1727-1734.

Sutton JR, Reeves JT, Wagner PD, Groves BM, Cymerman A, Malconian MK, Rock PB, Young PM, Walter SD & Houston CS. (1988). Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *J Appl Physiol* 64, 1309-1321.

Verges S, Bachasson D & Wuyam B. (2010). Effect of acute hypoxia on respiratory muscle fatigue in healthy humans. *Respir Res* 11, 109.

Wagner PD. (1996). Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol* 58, 21-50.

Wagner PD, Sutton JR, Reeves JT, Cymerman A, Groves BM & Malconian MK. (1987). Operation Everest II: pulmonary gas exchange during a simulated ascent of Mt. Everest. *J Appl Physiol* 63, 2348-2359.

Wasserman K. (1986). The anaerobic threshold: definition, physiological significance and identification. *Adv Cardiol* 35, 1-23.

Yoshitani K, Kawaguchi M, Miura N, Okuno T, Kanoda T, Ohnishi Y & Kuro M. (2007). Effects of hemoglobin concentration, skull thickness, and the area of the cerebrospinal fluid layer on near-infrared spectroscopy measurements. *Anesthesiology* 106, 458-462.

J Appl Physiol 112: 106–117, 2012.
First published October 27, 2011; doi:10.1152/japplphysiol.00388.2011.

"Live high–train low" using normobaric hypoxia: a double-blinded, placebo-controlled study

Christoph Siebenmann,[1] Paul Robach,[2] Robert A. Jacobs,[1,3] Peter Rasmussen,[1] Nikolai Nordsborg,[4] Victor Diaz,[1,3] Andreas Christ,[5] Niels Vidiendal Olsen,[6] Marco Maggiorini,[5] and Carsten Lundby[1]

[1]*Center for Integrative Human Physiology, Institute of Physiology, University of Zurich, Zurich, Switzerland;* [2]*Département Médical, Ecole Nationale des Sports de Montagne, Chamonix, France;* [3]*Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland;* [4]*Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark;* [5]*Intensive Care Unit, Department of Internal Medicine, University Hospital, Zurich, Switzerland; and* [6]*Department of Neuroanaesthesia, The Neuroscience Centre, Rigshospitalet, Copenhagen, Denmark*

Submitted 30 March 2011; accepted in final form 21 October 2011

Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M, Lundby C. "Live high–train low" using normobaric hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol* 112: 106–117, 2012. First published October 27, 2011; doi:10.1152/japplphysiol.00388.2011.— The combination of living at altitude and training near sea level [live high–train low (LHTL)] may improve performance of endurance athletes. However, to date, no study can rule out a potential placebo effect as at least part of the explanation, especially for performance measures. With the use of a placebo-controlled, double-blinded design, we tested the hypothesis that LHTL-related improvements in endurance performance are mediated through physiological mechanisms and not through a placebo effect. Sixteen endurance cyclists trained for 8 wk at low altitude (<1,200 m). After a 2-wk lead-in period, athletes spent 16 h/day for the following 4 wk in rooms flushed with either normal air (placebo group, $n = 6$) or normobaric hypoxia, corresponding to an altitude of 3,000 m (LHTL group, $n = 10$). Physiological investigations were performed twice during the lead-in period, after 3 and 4 wk during the LHTL intervention, and again, 1 and 2 wk after the LHTL intervention. Questionnaires revealed that subjects were unaware of group classification. Weekly training effort was similar between groups. Hb mass, maximal oxygen uptake (VO_2) in normoxia, and at a simulated altitude of 2,500 m and mean power output in a simulated, 26.15-km time trial remained unchanged in both groups throughout the study. Exercise economy (i.e., VO_2 measured at 200 W) did not change during the LHTL intervention and was never significantly different between groups. In conclusion, 4 wk of LHTL, using 16 h/day of normobaric hypoxia, did not improve endurance performance or any of the measured, associated physiological variables.

altitude; LHTL; performance; training

OVER THE PAST FIVE DECADES, endurance athletes have attempted to improve sea-level performance by means of altitude training. In the early 1990s, Levine and Stray-Gundersen (36) introduced the "live high–train low" (LHTL) strategy, where athletes reside and spend the majority of the day at moderate altitude while training closer to sea level. This paradigm aims for athletes to benefit from physiological adaptation to hypoxia, while avoiding the detrimental impact of hypoxia on high-intensity endurance training. After an initial study had provided results indicating that LHTL enhances aerobic performance in competitive runners (34), a bulk of follow-up studies confirmed these benefits across a variety of endurance disciplines (7, 50, 57, 59). None of them, however, used a double-blinded design, and thus it cannot be ruled out that the observed effects, especially on performance measures, were, at least in part, mediated by a placebo effect (11, 56). Our main aim with this study was therefore to investigate the effect of LHTL in a double-blinded, placebo-controlled study.

A further aim was to examine the effect of LHTL on exercise capacity in mild hypoxia. Some endurance disciplines, such as cross-country skiing, frequently perform competitions at moderate altitudes. However, even the mild hypoxia associated with these altitudes may decrease maximal oxygen uptake (VO_2max) by reducing arterial O_2 content (CaO_2) (22, 43), and this mechanism seems to be particularly pronounced in highly trained athletes (30, 31). It appears plausible that this detrimental effect of moderate altitude could be counterbalanced by measures that enhance CaO_2. Indeed, LHTL has previously been reported to not only normalize but also evenly enhance CaO_2 at an altitude of 2,340 m so that VO_2max was partially recovered (55). However, the study was conducted without a control/placebo group, and it thus remains unclear whether the LHTL intervention was superior to conventional training at low altitude. In the present study, we therefore performed additional VO_2max tests at a simulated altitude of 2,500 m to evaluate the impact of LHTL on exercise capacity in mild hypoxia with a placebo-controlled study design.

There has been much controversy as to the mechanisms underlying performance gains following LHTL (20, 35). In the initial LHTL study and the follow-up studies by the same group, performance gains were attributed to altitude-induced polycythemia and a concomitant increase in convective O_2 transport capacity (10, 34, 57). However, another subset of LHTL studies failed to reproduce the erythropoietic stimulation by LHTL and instead, observed improvements in exercise economy following LHTL (19, 52, 53). In an attempt to shed more light on the mechanisms underlying performance gains with LHTL, we evaluated both total Hb mass (Hb_{mass}) and exercise economy on five occasions before, during, and after the 4 wk of LHTL.

Thus the major aim of the present study was to determine changes in endurance performance of highly trained endurance athletes after 4 wk of LHTL (minimum 16 h/day at 3,000 m) using a double-blinded, placebo-controlled design. The hypotheses to be tested were: *1*) LHTL exerts a positive effect on endurance performance (VO_2max and time-trial performance),

Address for reprint requests and other correspondence: C. Lundby, Center for Integrative Human Physiology (ZIHP), Univ. of Zurich, Institute of Physiology, Office 23 H 6, Winterthurerstr. 190, 8057 Zürich, Switzerland (e-mail: carsten.lundby@access.uzh.ch).

which is based on altitude-dependent physiological adaptations and not exclusively on a placebo effect; *2*) LHTL increases VO_2max at moderate altitude; and *3*) potential performance gains after LHTL correlate with changes in Hb_{mass}.

METHODS

Subjects

Initially, 24 endurance athletes were recruited as subjects for the present study. However, due to personal reasons, five subjects withdrew participation in the last week before the study. Two further subjects did not show up at the onset of the study for unknown reasons. Finally, 17 highly trained endurance athletes living at or near sea level (16 males, 1 female, age 29 ± 6 years, height 179 ± 8 cm, body wt 69 ± 9 kg) from various countries in North America and Europe attended as subjects in the present study. All of them regularly participated in endurance competitions on at least national levels in disciplines related to cycling, i.e., road cycling, triathlon, cycle cross, and/or mountain bike. To prevent bias from previous altitude acclimatization, we excluded subjects who traveled to altitudes higher than 2,500 m within the last month before the study. All subjects gave written, informed consent to participate, and the study was approved by the local ethical boards (Kanton Zurich and Kanton Waadt, Switzerland). During the course of the experiment, one subject decided to withdraw participation for personal reasons, and hence, his data were not included into the analysis. The remaining 16 subjects all completed the study.

Study Design

At the onset of the study, subjects traveled to Prémanon, France (1,135 m), where they lived for 8 full wk at the Centre National de Ski Nordique, an accommodation of the French state, which is used by national endurance athletes for housing and training. This facility is equipped with fully furnished hypoxic rooms, in which athletes can comfortably live while being exposed to adjustable normobaric hypoxia. All of the experimental procedures of the study were performed at the hospital La Vallée (Le Sentier, Switzerland), located at an altitude of 1,020 m, ~25 km away from Prémanon. Subjects were transported from Prémanon to Le Sentier in mini-buses on experimental days.

The first 2 wk of the study served as a lead-in period, where subjects were familiarized to the natural environment, and baseline testing was performed. For the following 4 wk, the intervention period, subjects were assigned to spend a minimum of 16 h/day in one- to three-person hypoxic rooms containing either normobaric normoxia (placebo group, $n = 6$) or normobaric hypoxia (LHTL group, $n = 10$). The group classification was performed in a stratified but not randomized manner to provide optimal equality regarding physiological parameters and regular distribution of athletes of different disciplines. Consequently, the LHTL group was composed of three cross-cyclists, two triathletes, four road cyclists, and one mountainbiker, and the placebo group of two cross-cyclists, one triathlete, two road cyclists, and one mountain biker.

Three days prior to the study start, all rooms were controlled and calibrated with precision gases by the company who built the facilities (Fieldbrook, London, UK). Subjects were blinded toward the environmental condition to which they were exposed in their rooms. Furthermore, all investigators, except for the main investigator (who did not perform any measurements), were blinded toward the group assignment. All rooms had the required hypoxic equipment installed, and the air pumps were constantly turned on. O_2 fraction in each room was continuously monitored from two independent O_2 probes (T7OX-V, Oxygen CiTiceL, City Technology, Portsmouth, UK) connected to a control panel located in a room with restricted access. O_2 fraction was controlled by the main investigator 5 days/wk (this person staying in the control room overnight) and by a qualified person from the center, not involved in any measurement, during the 2 other days. In addition, O_2 fraction in each room was controlled twice daily via a portable O_2 sensor (Vandagraph VN2O2, Cambridge Sensotec, Cambridge, UK) by either the main investigator or the person from the center.

The LHTL group was exposed to a normobaric hypoxia equivalent to 2,500 m for the first 2 days/nights of the intervention period. Thereafter, the O_2 fraction was decreased, equivalent to 3,000 m. As a result, morning arterial O_2 saturation (SaO_2), estimated by pulse oximetry (NPB-290, Nellcor Puritan Bennett, Pleasanton, CA), was 92 ± 2% in the LHTL group (at 3,000 m) and 97 ± 1% in the placebo group ($P < 0.05$). Subjects were confined to their rooms from 20:00 to 07:00, from 08:00 to 10:00, and again from 16:00 to 19:00 during these 4 wk. However, they were always allowed to spend more time in their rooms if desired (but this was not recorded). The confinement was rigorously supervised by the main investigator 5 days/wk and by other investigators the remaining 2 days. For psychological reasons and to blind the subjects regarding group classification, all subjects were assigned to different roommates and/or rooms on a weekly basis.

During the last 2 wk of the study, the postintervention period, subjects were relieved of the room confinement and hypoxic exposure. These days had the purpose of monitoring how long potential effects of LHTL would last. However, the blinding was maintained for both subjects and investigators until the end of the study.

Five testing sessions were distributed over the 8 wk of the study. They were scheduled in the first 2 wk (BASELINE), after 3 wk in the LHTL intervention (W 3), on the last 3 days of the LHTL intervention (W 4), and 1 (W 1 POST) and 2 wk (W 2 POST) after termination of the intervention period. To obtain a solid baseline value and allow for test familiarization, all measurements (except for hypoxic VO_2max; see below in paragraph) were performed in duplicate during BASELINE, each test separated from its previous by at least 4 days. For the maximal exercise tests and the time trials, the better result of the two tests was adopted as a baseline value, whereas for the measurement of hematological parameters and cycling economy, the average value over the two BASELINE tests was used. In the following four testing sessions, all tests were performed only once, except during W 4, where hematological parameters were again evaluated twice (with calculation of the average value) and where hypoxic VO_2max was not tested. All other tests were performed once at this time point. The protocol with the scheduling and content of the five testing sessions is illustrated in Fig. 1.

Measurements

Hb_{mass}. Hb_{mass} was measured by a modified version of a carbon monoxide (CO) rebreathing technique (41). The subject first rested for 20 min in a semirecumbent position. Thereafter, 2 ml blood was sampled from an antecubital vein through a 20-G catheter and analyzed immediately in quadruplicate for *1*) percent carboxy-Hb (%HbCO) and Hb concentration ([Hb]) on a hemoximeter (ABL800, Radiometer, Copenhagen, Denmark) and *2*) hematocrit by the microhematocrit method (4 min at 13,500 rpm). After baseline collection and control, the subject first breathed 100% O_2 for 2 min to flush nitrogen from the airways. The breathing circuit (previously O_2 flushed) was then closed, and a bolus (1.5 ml/kg) of 99.997% chemically pure CO (CO N47, Air Liquide, Paris, France) was administrated and rebreathed for 8 min. At the end of the rebreathing period, another 2 ml blood sample was obtained and analyzed, following the same procedure. The change in %HbCO between first and second measurement was used for calculation of Hb_{mass}, taking into account the amount of CO remaining in the rebreathing circuit at the end of the procedure (2.2%) (8). Total red blood cell volume (RCV), blood volume, and plasma volume was derived from Hb_{mass}, [Hb], and hematocrit (8). All CO rebreathing tests were performed by the same operator. BASELINE and W 4 values reported here correspond to the average of duplicate measurements conducted on separate days within these testing ses-

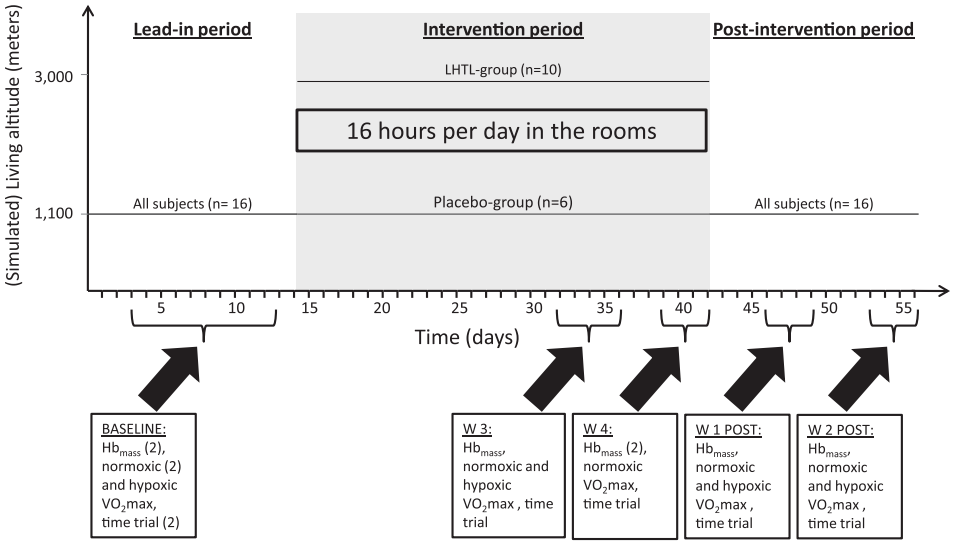

Fig. 1. Study protocol and testing sessions. The measurements performed in each testing session are listed; (2) indicates that a measurement was performed in duplicate within this session. LHTL, live high–train low; Hb_{mass}, total Hb mass; VO_2max, maximal oxygen uptake; BASELINE, first 2 wk; W 3, after 3 wk in the LHTL intervention; W 4, on the last 3 days of the LHTL intervention; W 1 POST and W 2 POST, 1 and 2 wk, respectively, after termination of the intervention period.

sions. The coefficient of variation for Hb_{mass}, assessed from duplicate BASELINE during the lead-in period and expressed as the percent typical error (i.e., SD of difference scores/$\sqrt{2}$), was 2.6%.

Urine erythropoietin. Total urine erythropoietin (Epo) concentration was determined using an ELISA designed to target both endogenous and recombinant Epo isoforms (Quantikine IVD Epo ELISA, R&D Systems, Minneapolis, MN). Morning urine (20 ml) was collected (BD Falcon Tubes, BD Biosciences, Rockville, MD) under visual inspection before breakfast and placed at −80°C until analyzed. Urine samples were collected on repeated occasions during the study. Three time periods are reported here: *1*) during the lead-in period (BASELINE); *2*) after 2–6 days of LHTL intervention; *3*) after 20–27 days of LHTL. Subjects were fully informed that the purpose of urine sampling was to screen for potential doping (no signs of recombinant human Epo doping were found). Random collection explains why urine samples were not collected on the same day for all subjects.

VO_2max and cycling economy. VO_2max was tested on an electronically braked bicycle ergometer (Monark, Varberg, Sweden), and the athletes used their own shoes and pedals and a race saddle. The exercise protocol started with a warm-up period of 5 min at a workload of 150 W, followed by 5 min at 200 W, except for the female athlete, who warmed up at 100 and 150 W. Thereafter, the workload was increased by 25 W/min^{-1} until voluntary exhaustion. During the last minutes of the test, subjects were vigorously encouraged to perform to complete exhaustion, and the achievement of VO_2max was established by standard criteria in all tests (1). Subjects wore a face mask covering their mouth and nose for breath collection (Hans Rudolph, Kansas City, MO), and O_2 and carbon dioxide concentration in the expired gas was continuously measured and monitored as breath-by-breath values (Quark, Cosmed, Rome, Italy). The gas analyzers and the flowmeter of the applied spirometer were calibrated prior to each test.

After the test, athletes recovered for 90 min, and then a second VO_2max test was performed, during which subjects were breathing a hypoxic gas mixture corresponding to an altitude of 2,500 m (Alti-Trainer, SMTEC, Nyon, Switzerland). This test followed the same protocol as the previous, except that warm-up workloads were set at 100 W and 150 W (75 W and 125 W for the female athlete). A hypoxic trial was performed in each testing session, except during W 4.

After the test, breath-by-breath values were visually controlled and averaged over 30 s. The highest average value was determined VO_2max, and all of the other parameters were picked at the same time, except for maximal workload (W_{max}), which was calculated as $W_{max} = W_{compl} + 25 \times (t/60)$; W_{compl} is the last completed workload, and t is the number of seconds in the not-W_{compl}.

To determine cycling economy, we assessed VO_2 during submaximal steady-state cycling. For this purpose, the breath-by-breath values of the last minute of the higher warm-up workloads (i.e., 200 W in normoxia and 150 W in hypoxia) were averaged. Since the only female athlete warmed up at lower workloads, she was not included into the evaluation of cycling economy.

Arterial blood sampling. After local anesthesia with 2% lidocaine, a 20-gauge catheter (model 80115.09R, Vygon Laboratories, Ecouen, France) was inserted percutaneously using the Seldinger technique into the radial artery. Arterial blood was sampled anaerobically in heparinized syringes and analyzed immediately for [Hb], SaO_2, and lactate concentration by means of the ABL800. Arterial samples were obtained during incremental exercise to exhaustion (VO_2max) on two separate occasions, i.e., at BASELINE and at W 3 during the normoxic and hypoxic exercise trials. Samples were collected at 200 W (normoxia) or 150 W (hypoxia) and at exhaustion. In the placebo group, one subject did not undergo catheterization during W 3. In the LHTL group, catheterization was not possible for one subject at BASELINE and for two subjects at W 3. Arterial blood sampling was thus conducted in $n = 5$ (placebo) and $n = 7$ (LHTL).

Time-trial performance. To evaluate exercise performance in a scenario similar to competitions, subjects performed a time trial using their own personal bike mounted on an electrically braked cycle trainer (Fortius Virtual Reality Trainer, Tacx, Rotterdam, Netherlands). The combination with commercially available software allowed for the simulation of a predefined route on a portable computer. We selected the final section of the real Milan-San Remo race with a length of 26.15 km. This route consists of a first flat part (4.8 km, average incline 0.23%), a first climb (5.5 km, 4.19%), a downhill part (3.1 km, −6.79%), a second flat part (8.6 km, 0.25%), and the final climb (4.15 km, 3.13%).

Athletes' body weight was measured immediately before each trial for software-based calculation of the appropriate resistance. After warming up for a minimum of 10 min, athletes could rest again and start the test whenever they felt ready. To achieve resemblance to actual time-trial cycling competitions, subjects were free to manually change gears and drink and eat ad libitum and allowed visual feedback on the computer screen, which displayed their speed and covered distance. To avoid potential influence from the pressure in the rear tire on the recorded speed/time, the rear wheel of each bike was fitted with a power meter (Powertap Elite+, CycleOps, Madison, WI), and power output over the whole course was measured as outcome variable. Subjects were vigorously encouraged during all tests.

Personal Training and Monitoring

Subjects were instructed to follow their training habits and to keep training intensity and volume as constant as possible throughout the 8 wk. Training was monitored using heart-rate monitors (Polar, Suunto, or Garmin). The investigators advised some athletes on a few occasions to either increase or decrease training volume and/or intensity. Training monitoring did not include experimental exercise tests (which were similar for all athletes) or training time spent at or below 55% of maximal heart rate. Furthermore, subjects frequently rode back from the hospital to the housing facility after testing sessions, and this training time (1 h on average, low to medium intensity) was not recorded either. During *weeks 3* and *4*, subjects were asked to reduce training the day before the exercise tests to minimize the influence of fatigue.

Sport Nutrition and Iron Supplementation

During the entire study, subjects were provided ad libitum access to commercially available energy drinks and carbohydrate and protein bars (Sponser, Wollerau, Switzerland). The concentrations of ferritin and soluble transferrin receptor (sTfR) were determined by immunoturbidimetric assay on a Hitachi 911 automatic analyzer (Boehringer, Mannheim, Germany) in duplicates during the lead-in phase and demonstrated that no subject was iron deficient at the time of entering the study; the mean value was 80.6 ± 11.9 (range from 62 to 102) ng/ml^{-1} for ferritin and 2.5 ± 0.3 (range from 2.0 to 3.1) $mg/1^{-1}$ for sTfR. To prevent any bias from iron deficiency, all subjects were supplemented with a daily oral intake of 256 mg dried ferrous sulfate (Tardyferon 80 mg, Pierre Fabre, Australia), starting upon arrival and continuing until the end of the intervention period.

Efficacy of the Blinding Process

The efficacy of the blinding process was evaluated during the intervention period by questionnaires in which subjects were repeatedly asked to state whether they believed to be living in normoxia or hypoxia.

Statistics

Statistical evaluation of the data was performed by running a two-way ANOVA with repeated measurements, combined with a Tukey post hoc test for multiple comparisons. The statistical software was the commercially available program SigmaPlot 5.0. The data are presented as mean values ± SD. A P value <0.05 was considered statistically significant. A maximum likelihood ANOVA determined whether subject blinding was successful.

RESULTS

Training Effort and Efficacy of the Blinding Procedure

Figure 2 illustrates training volume and intensity in both groups. No difference in total exercise time or heart-rate distribution was observed between the two groups at any point. Although training intensity was well preserved, the recorded training volume was decreased in both groups in the beginning

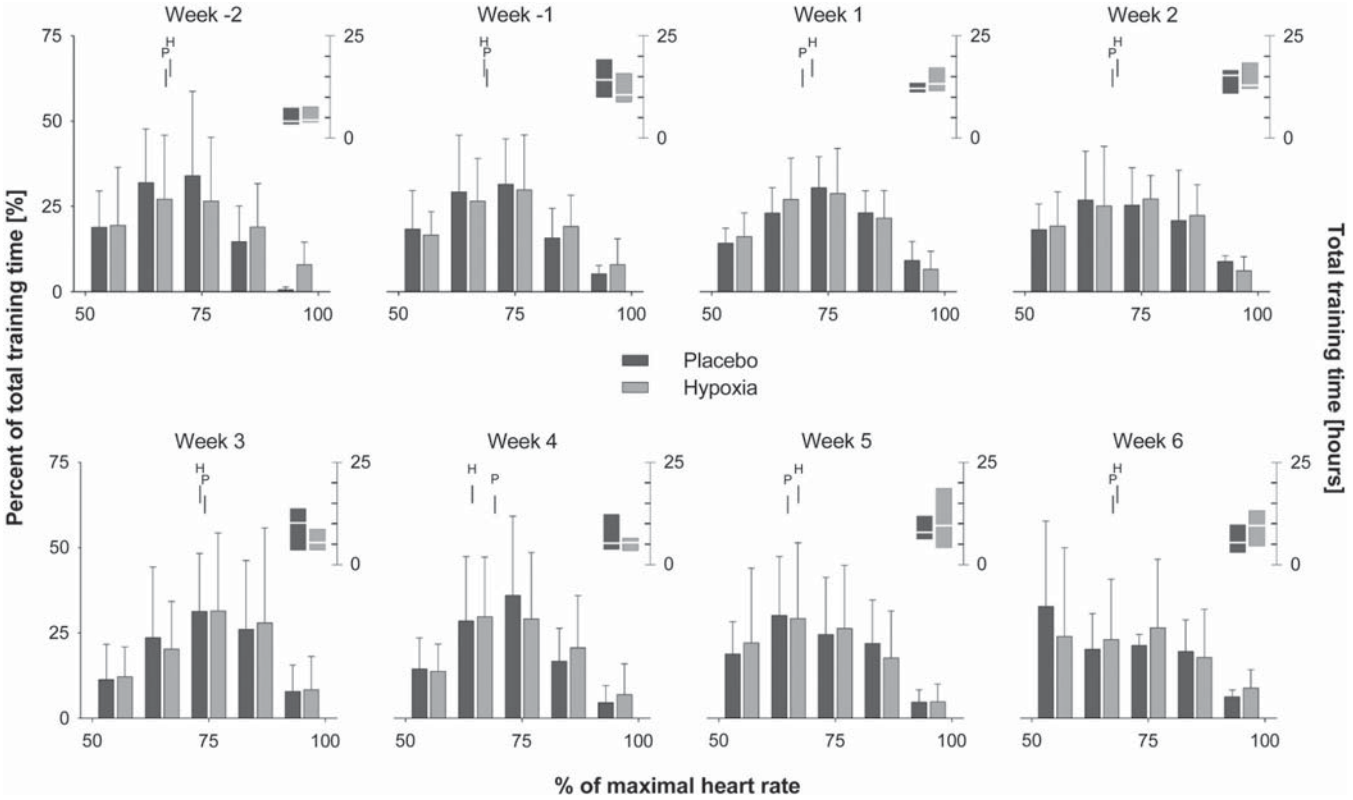

Fig. 2. Training. Training intensity (heart rate) and time (insets) over the course of the 8-wk study. In week-2 the volunteers arrived to the research facility, and values only represent 4 days. Exercise effort during the experiments and subsequent bike rides back to the housing facilities are not accounted for in this figure (as it can be assumed to be the same in both groups). Values for exercise intensity are weighted as means with SD and for the total training time, median with 25% quartiles. P and H with short lines indicate mean weighted heart-rate average for placebo and LHTL group, respectively. No differences were observed across time and study group with respect to exercise intensity, whereas the measured total training time decreased significantly with time in both groups but with no difference between the 2 groups.

Table 1. *Efficacy of subject blinding*

Days of LHTL	Guessed right	Guessed wrong	Could not decide
4	8 (50%)	3 (19%)	5 (31%)
8	7 (44%)	6 (38%)	3 (19%)
11	5 (31%)	4 (25%)	7 (44%)
18	7 (44%)	6 (38%)	3 (19%)
25	8 (50%)	4 (25%)	4 (25%)
28	4 (25%)	4 (25%)	8 (50%)

Number and fraction of subjects, able or unable to determine whether exposed to hypoxia or not. Time effect was not significant ($P = 0.639$). "Guessed wrong" and "Could not decide" answers were pooled. The maximal likelihood for the subjects to guess right was $P = 0.30$. The maximal likelihood for them to guess wrong or could not decide was $P = 0.06$. LHTL, live high–train low.

and toward the end of the study. This was primarily related to the high frequency of testing days in these weeks (exercise tests and the subsequent bike ride from the hospital to the facilities were not included into the monitoring of training effort) and the tapering day before, on which subjects were instructed to avoid long training sessions.

The results of the questionnaires evaluating the blinding process are presented in Table 1. The large variation, with only one-fourth of the subjects guessing right at the end of the LHTL period (equal to the number of subjects guessing wrong), indicates that the blinding process was successful, and subjects were unaware of group classification.

Hematological Parameters

Hematological parameters are summarized in Table 2 and Fig. 3. Despite a higher reticulocyte count ($P < 0.05$) in the LHTL group (17.4 ± 2.8%) than in the placebo group (12.7 ± 3.3%) at the end of the intervention period, Hb_{mass} was not affected and remained unchanged in both groups (Fig. 3, *A* and *B*). Furthermore, neither [Hb] nor hematocrit revealed a statistically significant difference between the two groups. However, during and after the LHTL intervention, both groups experienced a marked hemoconcentration with a parallel decrease in total blood volume and plasma volume and a concomitant increase in [Hb] and hematocrit (Table 2).

At W 4—thus at the end of the LTHL intervention—an increase in Hb_{mass}, which exceeded the typical error of 2.6% for the CO rebreathing procedure, was present in five of 10 subjects within the LHTL group, whereas in three subjects, Hb_{mass} had decreased by >2.6% (Fig. 3*C*). The corresponding individual changes in the placebo group are illustrated in Fig. 3*D*.

No change in urine Epo was detected across time in the placebo group, with values of 26.5 ± 13.9 ng/l^{-1} at BASELINE, 26.9 ± 14.3 ng/l^{-1} after 2–6 days, and 27.0 ± 15.2 ng/l^{-1} after 20–27 days of placebo exposure. In the LHTL group, urine Epo was higher ($P < 0.05$) after 2–6 days of LHTL exposure (35.7 ± 26.8 ng/l^{-1}) than at BASELINE (20.8 ± 15.5 ng/l^{-1}) but had returned to levels similar to BASELINE after 20–27 days of LHTL exposure (28.7 ± 20.2 ng/l^{-1}). The relative increase in urine Epo between BASELINE and short-term hypoxic exposure (*days 2–6*) was found to be higher in the LHTL than in the placebo group ($P < 0.05$). However, absolute values at 2–6 days did not differ significantly between groups, and furthermore, there was no correlation between hypoxia-induced changes in urine Epo and the corresponding variations in either reticulocyte count or Hb_{mass} at any time point (results not shown).

Maximal Exercise Capacity

VO_2max is illustrated in Fig. 4, and Table 3 summarizes further cardiorespiratory parameters obtained during maximal bicycle-ergometer exercise. The 8 wk of training increased VO_2max, expressed in ml/min^{-1}, by an average of 1.0 ± 3.8% in all subjects ($P = 0.06$). However, VO_2max remained unaffected by the intervention in the LHTL group and did not differ between groups at any time point. At W 4—thus at the end of the LHTL intervention—VO_2max was increased by 2.0 ± 1.6% in the placebo group and 0.0 ± 2.8% in the LHTL group ($P = 0.50$ for factor "time", and $P = 0.65$ for interaction between factors time and "group"). Correlation analysis indicated that in both groups, individual changes in VO_2max were not correlated to changes in either Hb_{mass} or [Hb].

Similar to the normoxic trials, VO_2max in hypoxia remained unaffected by the intervention (Fig. 4*C*) in both groups.

Arterial Blood Parameters During Exercise

Arterial blood parameters are summarized in Table 4, *A* (normoxic exercise) and *B* (hypoxic exercise at 2,500 m). The main result is that in both environmental conditions, 3 wk of LHTL exposure induced an increase in arterial [Hb], which supports the hemoconcentration observed in venous blood during CO rebreathing.

Cycling Economy

Cycling economy, expressed as average VO_2 during the higher warm-up workloads in normoxia (200 W) and hypoxia (150 W), is presented in Fig. 5. During the normoxic trials at W 1 Post (Fig. 5*A*), cycling economy at 200 W was decreased

Table 2. *[Hb], hematocrit, and intravascular volumes evaluated by carbon monoxide rebreathing*

	Placebo group ($n = 6$)					LHTL group ($n = 10$)				
	BASELINE	W 3	W 4	W 1 POST	W 2 POST	BASELINE	W 3	W 4	W 1 POST	W 2 POST
[Hb] (g/dl)	14.1 ± 0.3	15.2 ± 0.6*	15.3 ± 0.6*	15.0 ± 0.6*	15.1 ± 0.6*	13.9 ± 0.6	15.8 ± 0.6*	15.1 ± 0.7*	14.9 ± 0.7*	15.1 ± 0.7*
Hct (%)	43.0 ± 0.7	46.8 ± 1.6*	47.0 ± 1.4*	46.0 ± 1.5*	46.5 ± 1.3*	42.4 ± 1.8	47.8 ± 2.3*	46.4 ± 2.0*	45.5 ± 2.0*	46.2 ± 2.2*
RCV (l)	3.00 ± 0.33	3.01 ± 0.27	3.00 ± 0.28	3.00 ± 0.34	3.01 ± 0.30	2.84 ± 0.50	2.80 ± 0.50	2.89 ± 0.52	2.73 ± 0.50	2.76 ± 0.47
PV (l)	3.98 ± 0.37	3.43 ± 0.38*	3.39 ± 0.38*	3.49 ± 0.46*	3.47 ± 0.38*	3.86 ± 0.67	3.05 ± 0.44*	3.33 ± 0.57*	3.26 ± 0.54*	3.21 ± 0.52*
BV (l)	6.97 ± 0.70	6.44 ± 0.61*	6.38 ± 0.64*	6.45 ± 0.77*	6.49 ± 0.66*	6.69 ± 1.14	5.84 ± 0.91*	6.21 ± 1.05*	5.99 ± 1.00*	5.97 ± 1.00*

[Hb], venous Hb concentration; Hct, hematocrit; RCV, total red blood cell volume; PV, blood plasma volume; BV, total blood volume; BASELINE, first 2 wk; W 3, after 3 wk in the LHTL intervention; W 4, on the last 3 days of the LHTL intervention; W 1 POST and W 2 POST, 1 and 2 wk, respectively, after termination of the intervention period. Values are means ± SD; *$P < 0.05$ versus BASELINE; no significant differences between groups were observed.

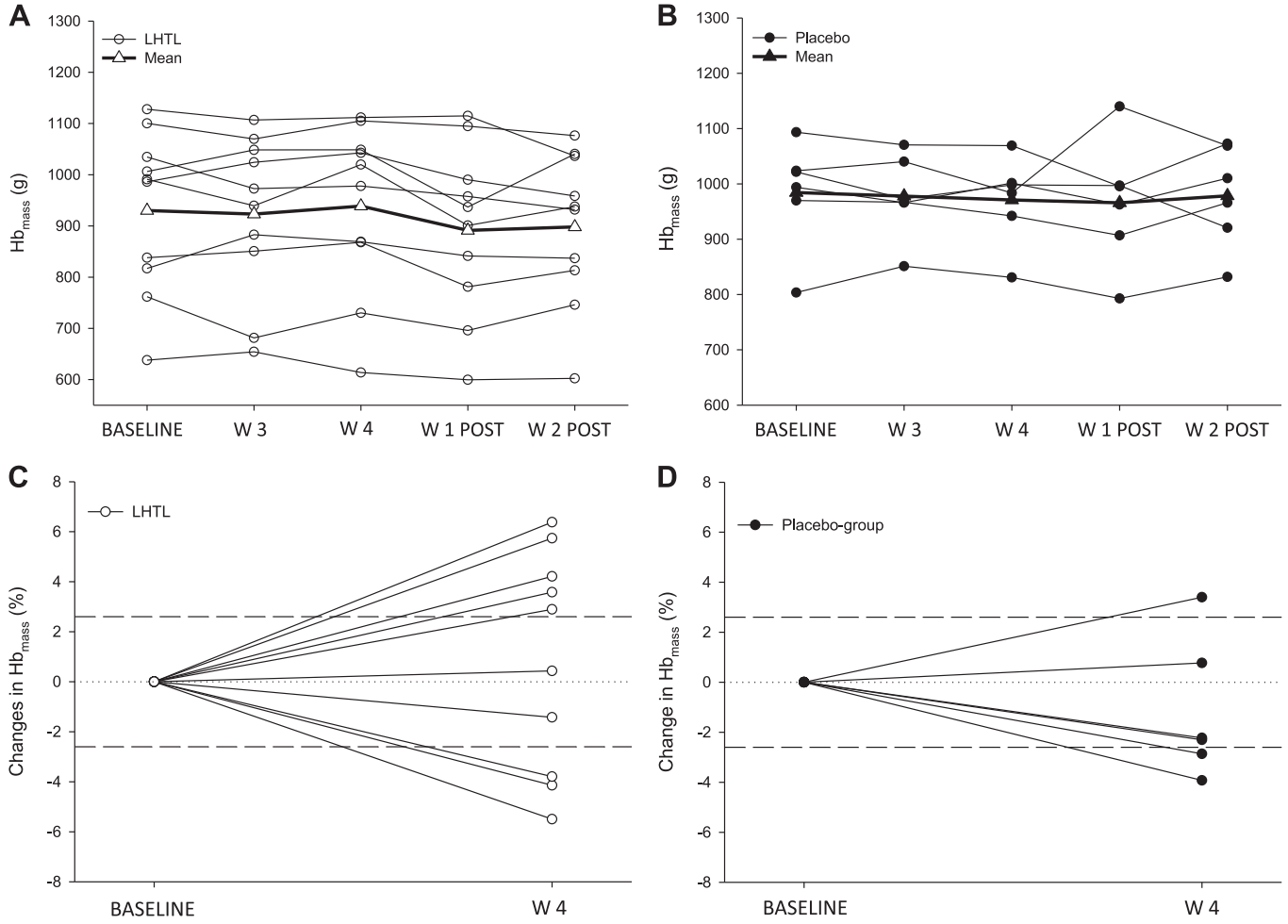

Fig. 3. Hb_{mass}. *A* and *B*: individual values for Hb_{mass} in the LHTL and placebo groups, respectively. Values at BASELINE and W 4 are average values of duplicate measurements. Group average values are represented by the triangles. Individual changes in Hb_{mass} from BASELINE to W 4 (i.e., the end of the intervention period) are depicted in *C* for the LHTL group and *D* for the placebo group. The dashed lines represent the typical error of the carbon monoxide rebreathing procedure in the present study (2.6%).

by 6.9% in all subjects ($P < 0.05$). However, the economy was never significantly different between the two groups. Economy evaluated during the hypoxic trials was unchanged throughout the entire study in both groups and never differed between groups (Fig. 5*B*).

Time-Trial Performance

Average power output during the 26.15-km time trial, expressed in Watt, is illustrated in Fig. 6. We observed a constant trend toward an increase in time-trial performance in all of our subjects over the course of the 8 wk, averaging 5% at the end of the study ($P = 0.12$). However, there was no effect of the LHTL intervention, and thus time-trial performance was always similar between the two groups. Separate analysis of five specific segments of the time trial (including climbs, descents, and flat sections) revealed no difference between groups either. At the end of the intervention period, i.e., at W 4, time-trial performance was increased by 5% in the placebo group and by 2% in the LHTL group ($P = 0.12$ for factor time, and $P = 0.33$ for interaction between factors time and group).

DISCUSSION

In the present study, we applied a double-blinded and placebo-controlled design to investigate the effects of LHTL on endurance performance of highly trained endurance cyclists. Our main finding is that LHTL did not affect Hb_{mass}, 26-km time-trial performance, VO_2max, or exercise economy in normoxia and moderate normobaric hypoxia, indicating that 4 wk of LHTL, using normobaric hypoxia, may not be superior to conventional endurance training.

Impact of LHTL on Aerobic Performance

Our findings are in contrast to the results of the initial LHTL study (34) and several follow-up studies, where improvements in VO_2max (7, 16, 50, 57) or endurance performance (57, 59) have been reported. This is intriguing, since we designed the LHTL intervention according to generally accepted recommendations, which for simulated altitude, range from 4 wk at 2,500–3,000 m (12–16 h/day) (61) to 18 days at 2,500 m (12 h/day) (44). Our total hypoxic dose exceeded 440 h at 2,500–3,000 m and was among the highest compared with studies

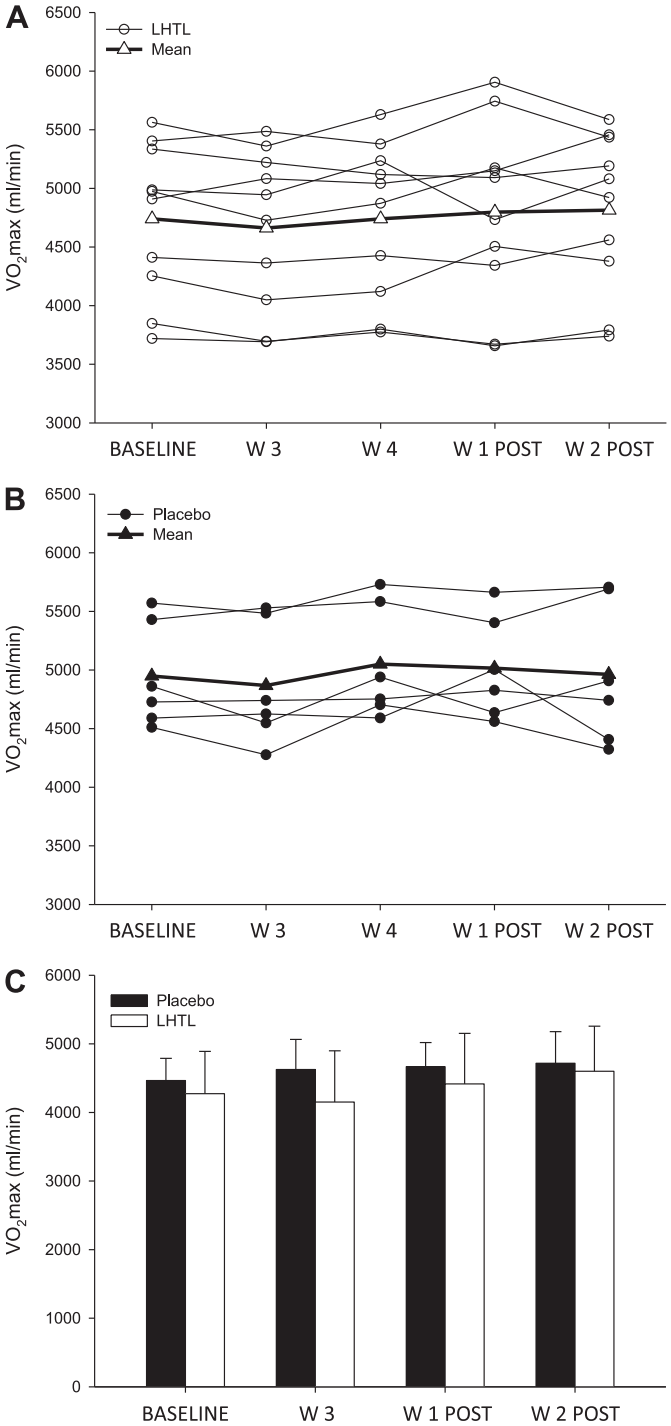

Fig. 4. VO_2max in normoxia (*A* and *B*) and normobaric hypoxia (*C*). *A* and *B*: in the normoxic tests (individual results for the LHTL group and the placebo group), the BASELINE values represent the better results of the duplicate measurement performed. Group average values are represented by the triangles in both figures. *C*: in the hypoxic tests, the columns represent mean values in both groups, and SDs are indicated by error bars. No significant changes or differences between groups were observed in either condition.

conducted in the past (7, 19, 33, 34, 44, 48–50, 52, 53, 58, 59, 61). Together with the strict control of the administrated atmosphere in the two groups, lower-pulse SaO_2 confirms hypoxemia in our subjects, whereas the elevated urine Epo levels and higher reticulocyte counts in the LHTL group suggest an accordingly physiological response. It is therefore unlikely that our results were associated with an insufficient hypoxic stimulus in the LHTL group. Furthermore, daily oral iron supplementation was provided, and the intake was controlled to avoid iron insufficiency. Finally, training intensity was well preserved in all groups over the course of the study, and the slight (nonsignificant) increase in VO_2max and time-trial performance in our already highly trained subjects indicates that the training stimulus was adequate. This raises the question as to why the LHTL group did not benefit from the 4 wk of the intervention. In this context, it is important to note that in the present study, the intervention failed to reproduce the physiological adaptations that have previously been proposed to underlie performance gains following LHTL, and accordingly, the lack of an ergogenic impact appears as a logical consequence. The absence of physiological adaptations to hypoxia in the LHTL group is discussed further below.

With the assessment of measures for aerobic performance using a double-blinded design, we had speculated to demonstrate that the ergogenic impacts of LHTL are independent of a placebo effect. Since earlier LHTL studies were always conducted with an unblinded design, it cannot be ruled out that a placebo effect may at least in part be responsible for the performance gains. This limitation may be particularly crucial in LHTL studies where the expected effects are minor (20). In fact, a recent meta analysis of 51 LHTL studies reported that the potential performance gains in elite athletes is ~4% (5). It is important to note in this context that with this large number of studies, the design and the quality are subject to great variation, which may have affected the outcome of the meta analysis. It nevertheless remains intriguing that an earlier study investigating the influence of a placebo effect on time-trial performance of competitive cyclists observed improvements that were in the same range as reported in the meta analysis (11). Furthermore, an open-label study design, as has been used in all previous LHTL studies, may not only have led to performance gains in the intervention group but may also have hampered the control group by a "nocebo effect" (3), i.e., the detrimental impact of low expectations and demotivation. This may have further widened the gap between subjects of the control and the intervention groups and ultimately created a statistically significant difference in some studies. To overcome this limitation, the present study was performed with a placebo-controlled design, and as illustrated in Table 1, our strategy to blind the subjects was successful. Nevertheless, since in our study, LHTL failed to improve aerobic performance, the present results cannot exclude that the performance benefits of LHTL reported in the past were, at least in part, related to a placebo effect. It could be retorted that if LHTL would benefit athletes by a placebo effect, our placebo group should have improved performance. Nevertheless, our subjects were always uncertain about their respective group classification, which may have prevented a potential placebo effect, as it may occur in open-label studies.

In summary, our study provides no indication for LHTL, using normobaric hypoxia, to improve time-trial performance or VO_2max of highly trained endurance cyclists more than conventional training. Given the considerable financial and

Table 3. *Workload and respiratory parameters during maximal cycling exercise*

	Placebo group (n = 6)					LHTL group (n = 10)				
	BASELINE	W 3	W 4	W 1 POST	W 2 POST	BASELINE	W 3	W 4	W 1 POST	W 2 POST
Workload (W)	411 ± 23	412 ± 31	422 ± 28	418 ± 30	417 ± 33	405 ± 59	400 ± 62	413 ± 57	398 ± 56	405 ± 55
VCO_2 (l/min)	5.35 ± 0.46	5.25 ± 0.45	5.59 ± 0.57	5.37 ± 0.51	5.39 ± 0.66	5.30 ± 0.75	5.01 ± 0.74*	5.25 ± 0.74	5.19 ± 0.84	5.30 ± 0.73
V_E/VO_2	37.3 ± 2.7	37.0 ± 2.2	36.6 ± 2.9	36.6 ± 2.0	37.7 ± 1.7	37.3 ± 4.0	37.8 ± 3.0	38.7 ± 3.3	36.7 ± 2.5	36.7 ± 2.8
V_E /VCO_2	34.4 ± 2.3	34.2 ± 1.1	33.1 ± 3.1	34.2 ± 1.8	34.4 ± 1.4	33.4 ± 3.8	35.1 ± 3.2	35.0 ± 3.4	33.7 ± 2.7	33.3 ± 2.9
RER	1.08 ± 0.04	1.08 ± 0.04	1.11 ± 0.02	1.07 ± 0.03	1.09 ± 0.03	1.12 ± 0.04	1.08 ± 0.04*	1.11 ± 0.06	1.08 ± 0.07	1.10 ± 0.06
V_E (l/min)	187 ± 5	183 ± 13	188 ± 15	187 ± 15	188 ± 19	181 ± 35	180 ± 33	187 ± 32	180 ± 34	180 ± 27
F (min^{-1})	60 ± 8	59 ± 8	59 ± 5	58 ± 9	60 ± 4	58 ± 7	54 ± 6	58 ± 7	53 ± 4	54 ± 5

VCO_2, carbon dioxide (CO_2) output; VO_2, oxygen (O_2) uptake; V_E, minute ventilation; RER, respiratory exchange ratio (VCO_2/VO_2); F, respiratory frequency. Values are means ± SD; *$P < 0.05$ versus BASELINE; no significant differences between groups were observed.

logistic effort of performing a LHTL camp, this should be taken into consideration before recommending LHTL to elite endurance athletes.

Potential Mechanisms Underlying LHTL

In their initial LHTL study, Levine and Stray-Gundersen (34) related the aerobic improvements following LHTL to a 8% gain in RCV, which correlated (r = 0.37; $P = 0.02$) to the increase in VO_2max. They hence concluded that the mechanism underlying LHTL is an enhanced, convective O_2 transport capacity, secondary to erythropoiesis stimulated by hypoxia. This approach seems convincing, since convective O_2 transport is the primary limiting factor of VO_2max (32, 51) and because acute manipulations of blood O_2 carrying capacity entail changes in VO_2max accordingly (14, 18, 40). Nevertheless, although the hypoxic exposure in the present study conformed with the generally accepted recommendations (44, 61) and despite quantifying Hb_{mass} on five different occasions with duplicate measurements during BASELINE and 4 W, we failed to detect an increase in Hb_{mass} in the LHTL group. In both groups, however, we observed some individuals to increase or decrease Hb_{mass} by the >2.6% typical error associated to our measurement, but these were all in a range that can be explained by natural variations over time (42). Accordingly, the increase in [Hb] in both groups was not caused by erythropoiesis but by a reduction in plasma volume. The latter may have counteracted a potential ergogenic effect of a higher O_2 carrying capacity secondary to the enhanced [Hb] (27). This explanation is supported by the fact that individual changes in [Hb] were not correlated to changes in VO_2max.

The intriguing observation that Hb_{mass} remained unaffected raises the question as to why the hypoxic stimulus failed to reproduce a erythropoietic response similar to that observed by Levine and Stray-Gundersen (34). Since Hb_{mass} appears insensitive to a placebo effect, this finding cannot be explained by the applied study design, and accordingly, other explanations should be considered.

In the initial LHTL study (34), subjects lived at natural altitude, as opposed to the artificially created normobaric hypoxia used in the present study, and it is tempting to conclude that this might explain the different outcomes of the two studies. Since performing a LHTL camp at natural altitude is related to a considerable logistic effort and due to the geographical conditions not feasible in many countries, the use of normobaric hypoxia is an appealing alternative for athletes. However, this is based on the assumption that the response to altitude is solely dependent on O_2 partial pressure and unrelated to barometric pressure. This has been questioned recently (12), and several studies have reported slight differences between the effects of normobaric and hypobaric hypoxia (37, 46, 54). Most importantly, in the present context, the reduction in CaO_2 seems to be more marked in hypobaric than in equivalent normobaric hypoxia (54). For this reason, the recommended minimal "altitude"

Table 4A. *Arterial blood parameters during exercise in normoxia*

	Placebo group (n = 5)		LHTL group (n = 7)	
	BASELINE	W 3	BASELINE	W 3
SaO_2 (%)				
200 W	95.3 ± 1.2	94.5 ± 1.7*	93.8 ± 3.1	94.5 ± 2.6*
Exhaustion	92.5 ± 4.0	91.7 ± 3.4	88.7 ± 2.2	91.4 ± 2.68*
$[Hb]_{art}$ (g/dl)				
200 W	14.9 ± 0.4	15.2 ± 0.5	14.2 ± 0.6	15.5 ± 1.0*
Exhaustion	15.6 ± 0.7	16.3 ± 0.7	15.2 ± 0.5	16.7 ± 1.0*
$[La]_{art}$ (mmol/l)				
200 W	1.4 ± 0.5	1.6 ± 0.6	1.9 ± 0.9	1.7 ± 0.5
Exhaustion	17.4 ± 1.7	15.7 ± 2.7	18.7 ± 2.1	16.2 ± 3.8
pH_{art}				
200 W	7.43 ± 0.02	7.41 ± 0.02	7.42 ± 0.03	7.39 ± 0.07
Exhaustion	7.22 ± 0.03	7.22 ± 0.06	7.22 ± 0.05	7.20 ± 0.06

SaO_2, arterial O_2 saturation; $[Hb]_{art}$, arterial [Hb]; $[La]_{art}$, arterial lactate concentration; pH_{art}, arterial pH. Values are means ± SD; *$P < 0.05$ versus BASELINE.

Table 4B. *Arterial blood parameters during exercise in acute normobaric hypoxia (2,500 m)*

	Placebo group (n = 5)		LHTL group (n = 7)	
	BASELINE	W 3	BASELINE	W 3
SaO_2 (%)				
150 W	88.3 ± 2.9	88.4 ± 1.9	86.9 ± 3.9	89.7 ± 4.2
Exhaustion	86.7 ± 5.0	83.2 ± 5.3	79.5 ± 4.8*	84.5 ± 5.2†
$[Hb]_{art}$ (g/dl)				
150 W	14.9 ± 0.6	14.9 ± 0.9	14.1 ± 0.6	15.5 ± 0.8†
Exhaustion	15.8 ± 0.5	16.2 ± 0.7	15.0 ± 0.6	16.3 ± 0.9†
$[La]_{art}$ (mmol/l)				
150 W	1.2 ± 0.5	1.4 ± 0.4	1.5 ± 0.6	1.7 ± 0.4
Exhaustion	16.2 ± 3.6	15.2 ± 3.4	17.5 ± 2.4	14.3 ± 2.7
pH_{art}				
150 W	7.45 ± 0.02	7.42 ± 0.05	7.46 ± 0.02	7.42 ± 0.08
Exhaustion	7.27 ± 0.07	7.20 ± 0.13	7.27 ± 0.05	7.28 ± 0.08

Values are means ± SD; *$P < 0.05$ versus BASELINE; †$P < 0.05$ versus placebo.

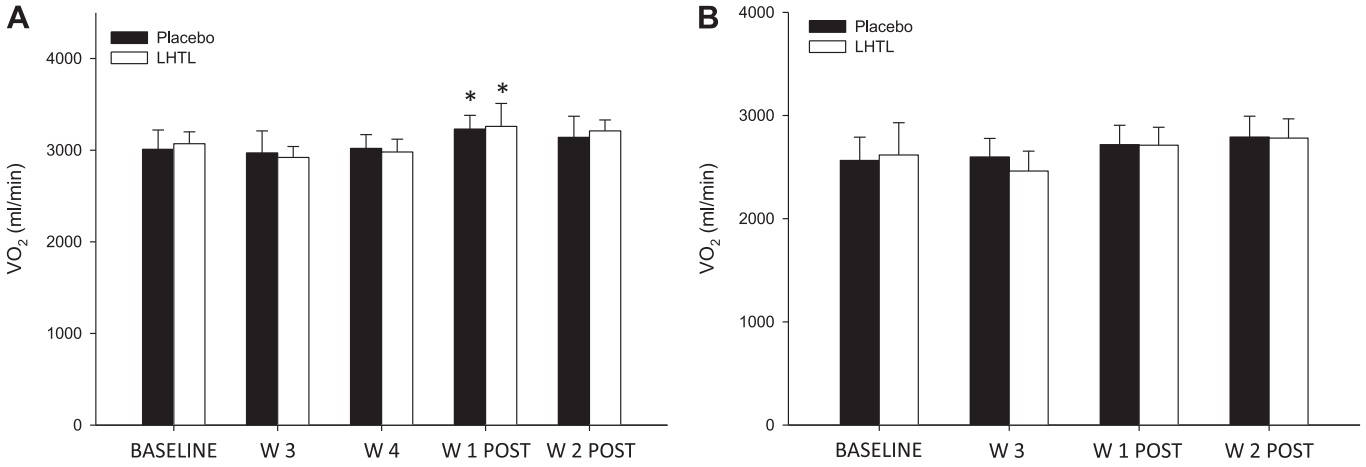

Fig. 5. Exercise economy during submaximal cycling in normoxia (*A*) and normobaric hypoxia (*B*). Economy in both conditions was evaluated as VO_2, averaged over the last minute of the higher warm-up workload (i.e., 150 W in hypoxia and 200 W in normoxia). The columns represent mean values in both groups, and SDs are indicated by error bars. The normoxic BASELINE values represent the average of the duplicate measurement performed. At W 1 POST, economy was slightly decreased in both groups compared with BASELINE (as indicated by *), but no significant differences between groups were observed.

for LHTL is also higher for normobaric hypoxia than for hypobaric hypoxia (44, 61), and this was taken into account in the present study. It should also be recognized that a previous study, conducted in the very same facilities, applied an equal degree of normobaric hypoxia and observed a pronounced increase in Hb_{mass} in the intervention group (7). We therefore conclude that neither the form nor the degree of hypoxia applied in the present study can explain the absence of a erythropoietic impact in the LHTL group.

A further potential explanation may be the loss of plasma volume, which was observed in both groups and accordingly, not a response to hypoxic exposure per se. Although the increase in urinal Epo concentration in the LHTL group speaks against it, the resulting hemoconcentration might have suppressed Epo synthesis and therefore, counteracted a potential erythropoietic stimulus in the LHTL group (4). We can indeed not explain the observed hemoconcentration, but it might have been a consequence of using normobaric hypoxia, which required our subjects to reside inside the hypoxic rooms for a considerable part of the day and thus limited their involvement in regular daily activities. It may be argued that these unusually long periods of inactivity might have triggered a reaction similar to that observed during bed-rest confinement, where a loss of plasma volume has been reported (23). Since the impact of room confinement in elite athletes remains uncertain, a potential effect on the plasma volume contraction in our subjects cannot be excluded, although the observation that plasma volume did not normalize after the end of the intervention period argues against it.

Although the absence of an increase in Hb_{mass} in our study was unexpected, it is partially supported by a retrospective analysis of the original data of Levine and Stray-Gundersen and coworker (10), where an increase in RCV was observed only in those subjects who also experienced an increase in endurance performance, which corresponded to 55%. This indicates a considerable interindividual variation in the response to LHTL, which is supported further by other studies reporting that the increase in serum Epo during prolonged

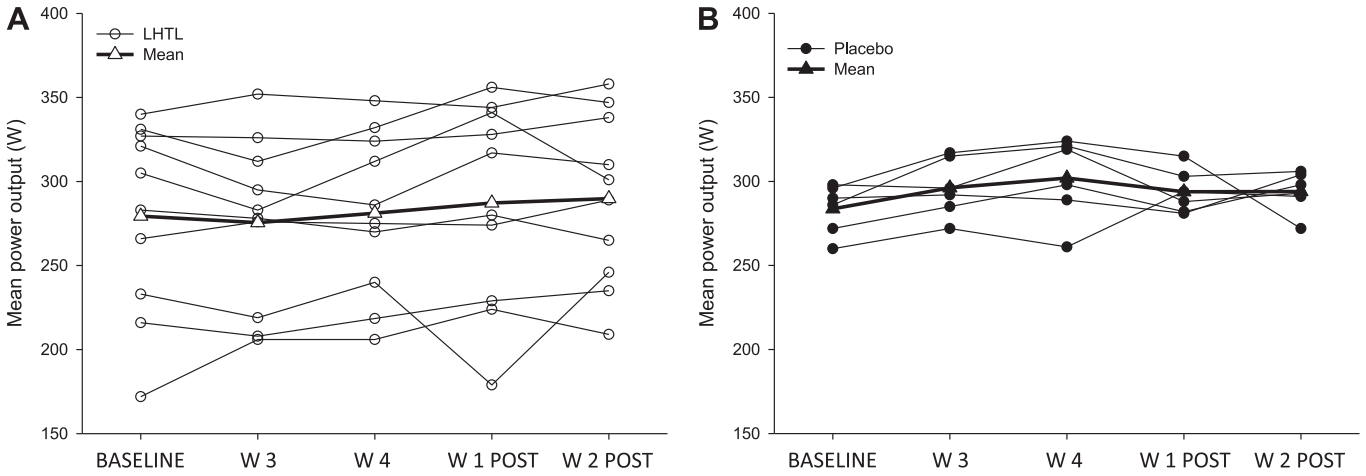

Fig. 6. Time-trial performance is expressed as mean power output over a 26.15-km-simulated time trial. Individual results of the subjects in the LHTL group (*A*) and the placebo group (*B*) are presented. The BASELINE values represent the better results of the duplicate measurement performed. Group average values are represented by the triangles in both figures. No significant changes or differences between groups were observed over the course of the study.

exposure to high altitude is variable by a factor >40 between individuals chronically exposed to very high altitude (45) or from −41 to 400% in subjects acutely exposed to moderate hypoxia (17). It thus appears that in LHTL studies, the presence of an effect on Hb_{mass} crucially depends on the random composition of the subject groups. This may, in combination with differences in study design, be the reason for the various outcomes of previous LHTL studies regarding gains in Hb_{mass}/RCV and perhaps also explain the lack of an increase in Hb_{mass} in the present study. In this context, it could be considered whether the already high BASELINE Hb_{mass} in our subjects could have contributed to our negative findings. Indeed, the BASELINE values for Hb_{mass} and RCV in our subjects were considerably higher than those reported in previous LHTL studies, demonstrating the most marked erythropoietic effects (7, 34, 59). The high Hb_{mass} may have blunted a potential erythropoietic response compared with less-trained subjects with accordingly lower Hb_{mass}. This, however, needs further research to be confirmed.

In summary, while the results of some earlier studies (7, 34, 53, 57, 59) indicate that LHTL may stimulate erythropoiesis in some athletes, our results demonstrate that this response is not certain in all subjects, which is probably explained by different individual responses to hypoxia (25). Coaches and athletes should be aware of this issue and remember that an individual's erythropoietic response to LHTL is, to date, difficult to predict.

The other common explanation for LHTL to increase performance is related to skeletal muscle adaptations to hypoxia (20). Gore and coworkers (19, 52, 53) base this on the observation of a higher exercise economy or muscle-buffer capacity (19) following LHTL. Exercise economy is, next to VO_2max and the percentage of VO_2max at which exercise is performed, one of the main components that determine the velocity in endurance events (13, 24, 26). Hence, an increased economy following LHTL would likely have improved our subjects' performance in the time-trial tests. However, we found no indications for LHTL to affect whole-body VO_2 during submaximal cycling performed on numerous occasions. Although an unchanged economy following LHTL has been reported previously (39), it is difficult to explain the difference with the studies in which a reduced submaximal exercise VO_2 has been observed (19, 28, 29, 52, 53). Nevertheless, none of these studies provides an objective description of training intensity, which was either controlled by subjective feedback from the athletes (19, 52) or not at all (28, 29, 53). It is therefore appealing to speculate that the open-label treatment and a concomitant placebo effect motivated LHTL subjects to train harder than their control counterparts, which in turn, may have improved running economy (15).

In summary, the present results provide no indication for LHTL to enhance whole-body exercise economy to an extent that improves aerobic performance. This is in line with our primary finding that LHTL did not enhance any of the parameters that usually determine endurance performance.

Effect of LHTL on VO_2max in Hypoxia

Winter sports, as well as disciplines from other sports, regularly hold competitions at altitudes ~2,500 m. Even the mild hypoxia associated with such altitudes impairs VO_2max by reducing CaO_2 (22, 43), and this appears to be pronounced in highly trained individuals (30, 31). Athletes may attenuate this undesirable effect by means that stimulate erythropoiesis and thus normalize arterial blood O_2 content. Accordingly, preliminary acclimatization may partly restore VO_2max in moderate hypoxia (2, 9). Furthermore, the ergogenic effect of erythropoietic stimulation by recombinant human Epo treatment is pronounced when exercise is performed at moderate altitude (47). Consequently, preliminary altitude exposure may be crucial for the success in endurance races at altitude, particularly on an elite level, where differences in the range of 0.5% may determine the outcome (21). However, during the time span required for altitude acclimatization, athletes have to acquiesce the detrimental impact of hypoxia on high-intensity training quality (6), which may counteract a potential advantage obtained by erythropoiesis. LHTL appears to be an elegant approach to avoid this dilemma, since it may allow for altitude acclimatization while training intensities can be maintained. This was supported by a recent study, where 3 wk of LHTL partially restored VO_2max at an altitude of 2,340 m (55). However, this study was designed without a placebo or even a control group, and thus the contribution of a placebo effect and/or training effect cannot be ruled out.

In contrast to these previous findings, the present data do not support the hypothesis that LHTL partly normalizes VO_2max at the simulated altitude of 2,500 m. The observation that VO_2max in moderate hypoxia was unchanged after LHTL, in spite of a significant increase in [Hb], suggests that either maximal cardiac output or O_2 extraction or both were reduced at that time. We conclude that for competitions at moderate altitude, precedent LHTL does not provide an athletic advantage, at least in elite cyclists.

Limitations

As discussed earlier, we observed a significant hemoconcentration in both groups, which may have suppressed Epo in the LHTL group (4). Although an Epo response was suggested in the urinal samples obtained from the LHTL group, it has to be emphasized that urinal measurements provide neither the temporal nor quantitative validity of blood measurements (38). Accordingly, we can only draw limited conclusions regarding the contribution of the hemoconcentration to our negative findings.

Another potential limitation is related to the training prescription for the LHTL group, as subjects were instructed to maintain training as constant as possible. Although this has not been scientifically confirmed, a slight reduction of training effort at the onset of a LHTL camp may be preferable in practice to avoid overtraining. However, since we considered the blinding of our subjects toward the group classification most relevant, we could not adapt the training prescriptions to the (simulated) living altitude. Nevertheless, we are convinced that this issue did not mask any effects of the intervention, since in previous studies in which ergogenic effects of LHTL were observed, training efforts were also equal between groups (7, 34, 50). Furthermore, since our subjects were competing in various disciplines and different countries, the present study included both—athletes preparing for an upcoming season (n = 10) and others that had finished their season prior to the study (n = 6). To avoid potential bias from this issue, we equally distributed the athletes of different disciplines, as well

as pre- and postseason athletes, over both groups and clearly instructed them to maintain training effort throughout the study. Nevertheless, we cannot exclude a slight influence of this issue on performance/motivation of our subjects, and the resulting noise of our measurements may have covered small effects of the intervention.

It should also be considered in this context whether the training effort of our subjects was sufficient to prevent detraining, which may also have masked an ergogenic impact in the LHTL group. However, the positive development of time-trial performance and VO_2max in our already highly trained athletes speaks against this.

It is important to note that our protocol did not allow subjects to schedule a pronounced recovery period prior to exercise tests, which is in contrast to preparation for an actual competition. To avoid disturbance of the subjects' training regime, we instructed them to perform only one easy training day prior to exercise tests, which is just a minimal form of tapering and might have slightly hampered performance in the tests. This was, however, the same for both groups, and it is thus doubtful that differences between groups were masked by fatigue.

Furthermore, it has to be considered whether the moderate elevation of our living and testing facilities contributed to the results of our study. However, a beneficial impact of a erythropoietic response would, if at all, be more pronounced in mild hypoxia (47). It may also be argued that the initial 2 wk at 1,135 m (the lead-in period) might have triggered a slight physiological response that has hampered a further adaption in the LHTL group during the intervention period. This is, however, unlikely, since even at the higher altitude of 1,600 m, no effects on [Hb] or hematocrit are usually observed (60). Most importantly, it must be acknowledged that in a previous LHTL study conducted in the same facilities and with a very similar protocol, the intervention improved the aerobic performance of runners (7). Hence, the natural elevation of the housing/testing facilities seems not to be a relevant factor for the outcome of our study.

Finally, we chose to perform the LHTL study using normobaric hypoxia, and thus we can only draw limited conclusion regarding LHTL at natural altitude. Future studies will have to establish whether athletes respond differently to these two forms of hypoxia.

Conclusion

Contrary to our hypothesis, the present results indicate that LHTL, using normobaric hypoxia, may not improve endurance performance at sea level or moderate altitude more than conventional training. Our results further suggest that the erythropoietic response to LHTL is subject to considerable interindividual variation and may be blunted in elite athletes, where baseline Hb_{mass}/RCV is already high. Finally, it is indicated that daily room confinement in elite athletes may lead to a reduction in plasma volume and thereby, counteract a potential erythropoietic response to normobaric LHTL. Future studies are encouraged to investigate the parameters underlying the interindividual variation in the response to LHTL and to compare the effects of LHTL at terrestrial altitude with those obtained with normobaric hypoxia.

GRANTS

Funding for this study was through grants obtained from Bundes Amt für Sport (BASPO; Switzerland), Team Denmark (Denmark), Ministère des Sports (France), and Institut National du Sport, de l'Expertise et de la Performance (France).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., M.M., and C.L. conception and design of research; C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., A.C., and N.V.O. performed experiments; C.S., P. Robach, R.A.J., P. Rasmussen, N.N., V.D., and N.V.O. analyzed data; C.S., P. Robach, and C.L. interpreted results of experiments; C.S. prepared figures; C.S. drafted manuscript; C.S. edited and revised manuscript; C.L. approved final version of manuscript.

REFERENCES

1. **American Thoracic Society, American College of Chest Physicians.** ATS/ACCP statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 167: 211–277, 2003.
2. **Bender PR, Groves BM, McCullough RE, McCullough RG, Huang SY, Hamilton AJ, Wagner PD, Cymerman A, Reeves JT.** Oxygen transport to exercising leg in chronic hypoxia. *J Appl Physiol* 65: 2592–2597, 1988.
3. **Benedetti F, Lanotte M, Lopiano L, Colloca L.** When words are painful: unraveling the mechanisms of the nocebo effect. *Neuroscience* 147: 260–271, 2007.
4. **Berglund B, Gennser M, Ornhagen H, Ostberg C, Wide L.** Erythropoietin concentrations during 10 days of normobaric hypoxia under controlled environmental circumstances. *Acta Physiol Scand* 174: 225–229, 2002.
5. **Bonetti DL, Hopkins WG.** Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med* 39: 107–127, 2009.
6. **Brosnan MJ, Martin DT, Hahn AG, Gore CJ, Hawley JA.** Impaired interval exercise responses in elite female cyclists at moderate simulated altitude. *J Appl Physiol* 89: 1819–1824, 2000.
7. **Brugniaux JV, Schmitt L, Robach P, Nicolet G, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Saas P, Chorvot MC, Cornolo J, Olsen NV, Richalet JP.** Eighteen days of "living high, training low" stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl Physiol* 100: 203–211, 2006.
8. **Burge CM, Skinner SL.** Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. *J Appl Physiol* 79: 623–631, 1995.
9. **Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD, Saltin B.** Why is VO2 max after altitude acclimatization still reduced despite normalization of arterial O2 content? *Am J Physiol Regul Integr Comp Physiol* 284: R304–R316, 2003.
10. **Chapman RF, Stray-Gundersen J, Levine BD.** Individual variation in response to altitude training. *J Appl Physiol* 85: 1448–1456, 1998.
11. **Clark VR, Hopkins WG, Hawley JA, Burke LM.** Placebo effect of carbohydrate feedings during a 40-km cycling time trial. *Med Sci Sports Exerc* 32: 1642–1647, 2000.
12. **Conkin J, Wessel JH 3rd.** Critique of the equivalent air altitude model. *Aviat Space Environ Med* 79: 975–982, 2008.
13. **di Prampero PE.** The energy cost of human locomotion on land and in water. *Int J Sports Med* 7: 55–72, 1986.
14. **Ekblom B, Wilson G, Astrand PO.** Central circulation during exercise after venesection and reinfusion of red blood cells. *J Appl Physiol* 40: 379–383, 1976.
15. **Franch J, Madsen K, Djurhuus MS, Pedersen PK.** Improved running economy following intensified training correlates with reduced ventilatory demands. *Med Sci Sports Exerc* 30: 1250–1256, 1998.
16. **Garvican LA, Pottgiesser T, Martin DT, Schumacher YO, Barras M, Gore CJ.** The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. *Eur J Appl Physiol*, 2010.
17. **Ge RL, Witkowski S, Zhang Y, Alfrey C, Sivieri M, Karlsen T, Resaland GK, Harber M, Stray-Gundersen J, Levine BD.** Determinants of erythropoietin release in response to short-term hypobaric hypoxia. *J Appl Physiol* 92: 2361–2367, 2002.

18. **Gledhill N.** Blood doping and related issues: a brief review. *Med Sci Sports Exerc* 14: 183–189, 1982.
19. **Gore CJ, Hahn AG, Aughey RJ, Martin DT, Ashenden MJ, Clark SA, Garnham AP, Roberts AD, Slater GJ, McKenna MJ.** Live high:train low increases muscle buffer capacity and submaximal cycling efficiency. *Acta Physiol Scand* 173: 275–286, 2001.
20. **Gore CJ, Hopkins WG.** Counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume. *J Appl Physiol* 99: 2055–2057, 2005.
21. **Hopkins WG, Hewson DJ.** Variability of competitive performance of distance runners. *Med Sci Sports Exerc* 33: 1588–1592, 2001.
22. **Hughes RL, Clode M, Edwards RH, Goodwin TJ, Jones NL.** Effect of inspired O2 on cardiopulmonary and metabolic responses to exercise in man. *J Appl Physiol* 24: 336–347, 1968.
23. **Iwasaki KI, Zhang R, Zuckerman JH, Pawelczyk JA, Levine BD.** Effect of head-down-tilt bed rest and hypovolemia on dynamic regulation of heart rate and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 279: R2189–R2199, 2000.
24. **Jacobs RA, Rasmussen P, Siebenmann C, Díaz V, Pesta D, Gnaiger E, Nordsborg NB, Robach P, Lundby C.** Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol*. First published September 1, 2011; doi:10.1152/japplphysiol.00625.2011.
25. **Jedlickova K, Stockton DW, Chen H, Stray-Gundersen J, Witkowski S, Ri-Li G, Jelinek J, Levine BD, Prchal JT.** Search for genetic determinants of individual variability of the erythropoietin response to high altitude. *Blood Cells Mol Dis* 31: 175–182, 2003.
26. **Joyner MJ, Coyle EF.** Endurance exercise performance: the physiology of champions. *J Physiol* 586: 35–44, 2008.
27. **Kanstrup IL, Ekblom B.** Acute hypervolemia, cardiac performance, and aerobic power during exercise. *J Appl Physiol* 52: 1186–1191, 1982.
28. **Katayama K, Matsuo H, Ishida K, Mori S, Miyamura M.** Intermittent hypoxia improves endurance performance and submaximal exercise efficiency. *High Alt Med Biol* 4: 291–304, 2003.
29. **Katayama K, Sato K, Matsuo H, Ishida K, Iwasaki K, Miyamura M.** Effect of intermittent hypoxia on oxygen uptake during submaximal exercise in endurance athletes. *Eur J Appl Physiol* 92: 75–83, 2004.
30. **Koistinen P, Takala T, Martikkala V, Leppaluoto J.** Aerobic fitness influences the response of maximal oxygen uptake and lactate threshold in acute hypobaric hypoxia. *Int J Sports Med* 16: 78–81, 1995.
31. **Lawler J, Powers SK, Thompson D.** Linear relationship between VO2max and VO2max decrement during exposure to acute hypoxia. *J Appl Physiol* 64: 1486–1492, 1988.
32. **Levine BD.** VO2max: what do we know, and what do we still need to know? *J Physiol* 586: 25–34, 2008.
33. **Levine BD, Stray-Gundersen J.** Dose-response of altitude training: how much altitude is enough? *Adv Exp Med Biol* 588: 233–247, 2006.
34. **Levine BD, Stray-Gundersen J.** "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 83: 102–112, 1997.
35. **Levine BD, Stray-Gundersen J.** Point: positive effects of intermittent hypoxia (live high:train low) on exercise performance are mediated primarily by augmented red cell volume. *J Appl Physiol* 99: 2053–2055, 2005.
36. **Levine BD, Stray-Gundersen J.** A practical approach to altitude training: where to live and train for optimal performance enhancement. *Int J Sports Med* 13, *Suppl* 1: S209–S212, 1992.
37. **Loeppky JA, Roach RC, Maes D, Hinghofer-Szalkay H, Roessler A, Gates L, Fletcher ER, Icenogle MV.** Role of hypobaria in fluid balance response to hypoxia. *High Alt Med Biol* 6: 60–71, 2005.
38. **Lonnberg M, Dehnes Y, Drevin M, Garle M, Lamon S, Leuenberger N, Quach T, Carlsson J.** Rapid affinity purification of erythropoietin from biological samples using disposable monoliths. *J Chromatogr A* 1217: 7031–7037, 2010.
39. **Lundby C, Calbet JA, Sander M, van Hall G, Mazzeo RS, Stray-Gundersen J, Stager JM, Chapman RF, Saltin B, Levine BD.** Exercise economy does not change after acclimatization to moderate to very high altitude. *Scand J Med Sci Sports* 17: 281–291, 2007.
40. **Lundby C, Robach P, Boushel R, Thomsen JJ, Rasmussen P, Koskolou M, Calbet JA.** Does recombinant human Epo increase exercise capacity by means other than augmenting oxygen transport? *J Appl Physiol* 105: 581–587, 2008.
41. **Lundby C, Thomsen JJ, Boushel R, Koskolou M, Warberg J, Calbet JA, Robach P.** Erythropoietin treatment elevates haemoglobin concentration by increasing red cell volume and depressing plasma volume. *J Physiol* 578: 309–314, 2007.
42. **Prommer N, Sottas PE, Schoch C, Schumacher YO, Schmidt W.** Total hemoglobin mass—a new parameter to detect blood doping? *Med Sci Sports Exerc* 40: 2112–2118, 2008.
43. **Pugh LG.** Athletes at altitude. *J Physiol* 192: 619–646, 1967.
44. **Richalet JP, Gore CJ.** Live and/or sleep high:train low, using normobaric hypoxia. *Scand J Med Sci Sports* 18, *Suppl* 1: 29–37, 2008.
45. **Richalet JP, Souberbielle JC, Antezana AM, Dechaux M, Le Trong JL, Bienvenu A, Daniel F, Blanchot C, Zittoun J.** Control of erythropoiesis in humans during prolonged exposure to the altitude of 6,542 m. *Am J Physiol* 266: R756–R764, 1994.
46. **Roach RC, Loeppky JA, Icenogle MV.** Acute mountain sickness: increased severity during simulated altitude compared with normobaric hypoxia. *J Appl Physiol* 81: 1908–1910, 1996.
47. **Robach P, Calbet JA, Thomsen JJ, Boushel R, Mollard P, Rasmussen P, Lundby C.** The ergogenic effect of recombinant human erythropoietin on VO2max depends on the severity of arterial hypoxemia. *PLoS One* 3: e2996, 2008.
48. **Robach P, Schmitt L, Brugniaux JV, Nicolet G, Duvallet A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV, Richalet JP.** Living high-training low: effect on erythropoiesis and maximal aerobic performance in elite Nordic skiers. *Eur J Appl Physiol* 97: 695–705, 2006.
49. **Robach P, Schmitt L, Brugniaux JV, Roels B, Millet G, Hellard P, Nicolet G, Duvallet A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV, Richalet JP.** Living high-training low: effect on erythropoiesis and aerobic performance in highly-trained swimmers. *Eur J Appl Physiol* 96: 423–433, 2006.
50. **Robertson EY, Saunders PU, Pyne DB, Aughey RJ, Anson JM, Gore CJ.** Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc* 42: 394–401, 2010.
51. **Saltin B, Strange S.** Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med Sci Sports Exerc* 24: 30–37, 1992.
52. **Saunders PU, Telford RD, Pyne DB, Cunningham RB, Gore CJ, Hahn AG, Hawley JA.** Improved running economy in elite runners after 20 days of simulated moderate-altitude exposure. *J Appl Physiol* 96: 931–937, 2004.
53. **Saunders PU, Telford RD, Pyne DB, Hahn AG, Gore CJ.** Improved running economy and increased hemoglobin mass in elite runners after extended moderate altitude exposure. *J Sci Med Sport* 12: 67–72, 2009.
54. **Savourey G, Launay JC, Besnard Y, Guinet A, Travers S.** Normo- and hypobaric hypoxia: are there any physiological differences? *Eur J Appl Physiol* 89: 122–126, 2003.
55. **Schuler B, Thomsen JJ, Gassmann M, Lundby C.** Timing the arrival at 2340 m altitude for aerobic performance. *Scand J Med Sci Sports* 17: 588–594, 2007.
56. **Sonetti DA, Wetter TJ, Pegelow DF, Dempsey JA.** Effects of respiratory muscle training versus placebo on endurance exercise performance. *Respir Physiol* 127: 185–199, 2001.
57. **Stray-Gundersen J, Chapman RF, Levine BD.** "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol* 91: 1113–1120, 2001.
58. **Stray-Gundersen J, Levine BD.** Live high, train low at natural altitude. *Scand J Med Sci Sports* 18, *Suppl* 1: 21–28, 2008.
59. **Wehrlin JP, Zuest P, Hallen J, Marti B.** Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. *J Appl Physiol* 100: 1938–1945, 2006.
60. **Weil JV, Jamieson G, Brown DW, Grover RF.** The red cell mass—arterial oxygen relationship in normal man. Application to patients with chronic obstructive airway disease. *J Clin Invest* 47: 1627–1639, 1968.
61. **Wilber RL, Stray-Gundersen J, Levine BD.** Effect of hypoxic "dose" on physiological responses and sea-level performance. *Med Sci Sports Exerc* 39: 1590–1599, 2007.

The role of haemoglobin mass on VO_2max following normobaric 'live high–train low' in endurance-trained athletes

Paul Robach,[1] Christoph Siebenmann,[2] Robert A Jacobs,[2,3] Peter Rasmussen,[2] Nikolai Nordsborg,[4] Dominik Pesta,[5] Erich Gnaiger,[5] Víctor Díaz,[3] Andreas Christ,[6] Julia Fiedler,[7] Nadine Crivelli,[7] Niels H Secher,[8] Aurélien Pichon,[9] Marco Maggiorini,[6] Carsten Lundby[2]

[1]Département Médical, Ecole Nationale de Ski et d'Alpinisme, site de l'Ecole Nationale des Sports de Montagne, Chamonix, France
[2]Zurich Center for Integrative Human Physiology (ZIPH), University of Zurich, Zurich, Switzerland
[3]Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland
[4]Department of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark
[5]Department of Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck Medical University, Innsbruck, Austria
[6]Intensive Care Unit DIM, University Hospital Zurich, Zurich, Switzerland
[7]Hôpital La Vallée, Le Sentier, Switzerland
[8]Department of Anaesthesia, The Copenhagen Muscle Research Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[9]Université Paris 13, Sorbonne Paris Cité, Laboratoire Réponses Cellulaires et Fonctionnelles à l'Hypoxie, Bobigny, France

Correspondence to
Professor Carsten Lundby, Center for Integrative Human Physiology (ZIHP), University of Zurich, Institute of Physiology, Office 23 H 6, Winterthurerstr. 190, Zurich 8057, Switzerland; carsten.lundby@access.uzh.ch

Accepted 9 June 2012

ABSTRACT

It remains unclear by which mechanism 'live high–train low' (LHTL) altitude training increases exercise performance. Haematological and skeletal muscle adaptations have both been proposed. To test the hypotheses that (i) LHTL improves maximal oxygen uptake (VO_2max) and (ii) this improvement is related to hypoxia-induced increases in total haemoglobin mass (Hb_{mass}) and not to improved maximal oxidative capacity of skeletal muscle, we determined VO_2max before LHTL and after LHTL, before and after the altitude-induced increases in Hb_{mass} (measured by carbon-monoxide rebreathing) had been abolished by isovolumic haemodilution. We obtained skeletal muscle biopsies to quantify mitochondrial oxidative capacity and efficiency. Sixteen endurance-trained athletes were assigned (double-blinded, placebo controlled) to ≥16 h/day over 4 weeks to normoxia (placebo, n=6) or normobaric hypoxia equivalent to 3000 m altitude (LHTL, n=10). Four-week LHTL did not increase VO_2max, irrespective of treatment (LHTL: 1.5%; placebo: 2.0%). Hb_{mass} was slightly increased (4.6%) in 5 (of 10) LHTL subjects but this was not accompanied by a concurrent increase in VO_2max. In the subjects demonstrating an increase in Hb_{mass}, isovolumic haemodilution elicited a 5.8% decrease in VO_2max. Cycling efficiency was altered neither with time nor by LHTL. Neither maximal capacity of oxidative phosphorylation nor mitochondrial efficiency was modified by time or LHTL. The present results suggest that LHTL has no positive effect on VO_2max in endurance-trained athletes because (i) muscle maximal oxidative capacity is not improved following LHTL and (ii) erythrocyte volume expansion after LHTL, if any, is too small to alter O_2 transport.

INTRODUCTION

The concept of 'live high–train low' (LHTL) was introduced to preserve the ability to train at a high intensity while acclimatising to hypoxia[1] and has repeatedly demonstrated a positive effect on VO_2max and sea-level performance.[2 3] Although the ergogenic effect of LHTL has not been unequivocally confirmed, LHTL is nonetheless considered a strategy to increase aerobic performance,[4–6] even if the potential improvement remains modest.[7]

Assuming that there is an effect of hypoxic exposure on VO_2max, the question arises which physiological mechanism(s) might be involved. Two hypoxia-related mechanisms were proposed, one being 'central', that is, an altitude-induced increase in total red blood cell volume (RCV)[4] and the other mechanism being an increase in skeletal muscle efficiency.[5] The rationale for an increased RCV (or total haemoglobin mass, Hb_{mass}) to be the main mechanism of LHTL is that (i) RCV is increased in proportion to the residing altitude,[8] (ii) when sufficient altitude 'time' is allowed, RCV is increased in most athletes participating in LHTL studies,[2 9–15] (iii) augmentation of RCV with autologous blood transfusion[16] or recombinant erythropoietin[17 18] increases aerobic performance and (iv) in those athletes for whom RCV is increased in response to LHTL, also VO_2max is increased.[19]

However, it is argued that attributing performance increases solely to enhanced erythropoiesis and RCV is inadequate,[5] since some LHTL studies have observed only minor change in RCV whereas at the same time running economy was improved,[20–22] suggesting increased muscular efficiency. Although the underlying mechanism has not been demonstrated, it may be that acclimatisation to altitude could improve oxidative enzyme activity and/or mitochondrial function, as shown after a hypoxic training intervention (live low–train high).[23] The suggestion of an effect of hypoxia on training-induced muscle adaptation is, however, not supported by muscle enzyme activity[24] or exercise economy data.[25]

Our study aimed to test two hypotheses: (i) normobaric LHTL improves VO_2max in endurance-trained athletes, and (ii) improvement in VO_2max following LHTL is related to hypoxia-induced increase in RCV (or Hb_{mass}) and not to increased maximal oxidative capacity of skeletal muscle. Our previous report indicates not only that LHTL did not improve VO_2max in our group of 10 athletes (hypothesis 1 was therefore not verified) but also that Hb_{mass} response to LHTL was variable, being positive in only half of the LHTL subjects.[26] The present paper examines the role of increased Hb_{mass} on VO_2max response to LHTL (hypothesis 2) by means of isovolumic haemodilution and reports on skeletal muscle mitochondrial function to gain insight into the mechanisms that may be responsible for the potential change in cycling efficiency.

METHODS

The present experiment is part of a double-blind, placebo-controlled study on LHTL reported in detail elsewhere.[26]

Sixteen (15 males and 1 female) trained cyclists (n=13) and triathletes (n=3), age 29±6 years (mean±SD), height 179±8 cm and body weight 69±9 kg participated in the study after giving oral and written informed consent. The study was approved by the Ethics Committee of Zurich (2010-066/0) and Vaud (215/10) (Switzerland), and conformed to the Declaration of Helsinki.

Study design

All subjects resided at the Centre National de Ski Nordique (1135 m, Prémanon, France) while all experimental procedures were performed at the neighbouring La Vallée hospital (1020 m, Le Sentier, Switzerland).

During the first 2 weeks (lead-in period) all subjects were exposed to the normal environment at Prémanon. For the following 4 weeks (intervention period), the subjects were assigned in a double-blinded, placebo-controlled manner to ≥16 h/day of either normobaric normoxia (placebo group, n=6) or normobaric hypoxia equivalent to 3000 m of altitude (LHTL group, n=10). Two testing sessions were scheduled for the present study, the first one during the lead-in period (baseline), and the second one during the last 3 days of the LHTL intervention period (W4).

Training

Details of the training protocol and results are reported elsewhere.[26] The subjects followed their own training habits and were instructed to keep the training intensity and load as stable as possible during the 6 weeks. Training was evaluated daily with a heart rate monitor, and in addition some of the subjects recorded their training load with power metres.[27] The average exercise intensity spent (time/week) at a given exercise intensity indicated equivalent training between groups. During weeks W3 and W4, the subjects were asked to taper in preparation for the test sessions. No differences were observed across time and study group with respect to exercise intensity, whereas the quantified total training time decreased significantly with regard to time in both groups but with no difference between the two groups.

Experimental protocols

Incremental exercise to exhaustion was carried out on an electronically braked cycle-ergometer (Monark E-839, Varberg, Sweden). The exercise protocol started with a warm-up period of 5 min at a workload of 150 W followed by 5 min at 200 W (100 and 150 W for the female athlete). Thereafter, the workload was increased by 25 W every minute until exhaustion. Attainment of maximal oxygen uptake was established by standard criteria.[28] Expired gas were continuously measured (Quark, Cosmed, Rome, Italy). Breath-by-breath values were averaged over 30 s and the highest averaged value was termed as VO_2max. Each subject performed two maximal exercise tests during the lead-in period. The largest VO_2max result between the two tests was used to express the baseline VO_2max.

Hb_{mass} was quantified by a modified version[29] of a carbon monoxide (CO)-rebreathing technique.[30] A detailed description of this procedure is given elsewhere.[26] Hb_{mass} was determined twice during the lead-in period (baseline) and twice during the fourth week of LHTL intervention (W4). Baseline and W4 values therefore correspond to the average of duplicate measurement. The coefficient of variation for Hb_{mass}, assessed from duplicate baseline during the lead-in period, and expressed as the percent typical error (ie, SD of difference scores/$\sqrt{2}$), was 2.6%.

Isovolumic haemodilution

To test the hypothesis that augmented RCV is the primary mediator of enhanced VO_2max following LHTL, maximal exercise was performed before and after isovolumic haemodilution at the end of the intervention period (W4). We withdrew the amount of extra red blood cells that was gained after LHTL by phlebotomy, and this volume was replaced by 6% hydroxyethyl starch (Voluven 6%, Fresenius Kabi, Bad Homburg, Germany) needed to achieve blood volume and RCV values similar to those measured at baseline. For each subject, the volume of blood to be withdrawn was calculated as $\Delta Hb_{mass}/[Hb]\times 100$, with ΔHb_{mass} being the change in Hb_{mass} with LHTL intervention and [Hb], resting haemoglobin concentration at W4 (measured by a Radiometer ABL800 FLEX analyser). Taking into account analytical variability for Hb_{mass} determination, phlebotomy was conducted only if the volume of blood to be removed exceeded 190 ml. Within 15 min after completion of the first exercise bout, blood was withdrawn into a blood bag (MRG6282L or MCG2272L, Macopharma, Mouvaux, France) positioned on a mixing scale (Docon, Möller Medical, Fulda, Germany), and then stored at 4°C. Hydroxyethyl starch infusion was initiated immediately after phlebotomy. In subjects for whom plasma volume decreased without a concomitant change in Hb_{mass}, only the starch infusion was performed. The subjects were blinded towards the procedure by placing their arm through a screen. In subjects for whom no manipulations were necessary, blood was withdrawn and reinfused to simulate these procedures. The second maximal test was initiated 2 h after the end of the first bout of exercise. After completion of the second exercise test, the subjects were either reinfused with their own blood, or infused with saline, again by using a blinded design. Blood sampling and infusion were performed via an 18-G catheter inserted in an arm vein.

Haematological measurements

Blood viscosity (in centipoises, cps) was measured within 3 h after blood collection in EDTA tubes with a cone/plate viscometer (Model DV-II, Brookfield Engineering Laboratories, Middleboro, Massachusetts, USA) with CPE40 spindle at three shear rates (45, 90 and 225/s).

Skeletal muscle biopsy and preparation of muscle fibres

Under local anaesthetics using the Bergström technique with suction, skeletal muscle biopsies (~20 mg) were obtained from the vastus lateralis muscle at baseline and at W4. The biopsy was dissected free of fat and connective tissue and thereafter measured for mitochondrial respiration, as previously reported.[31] Measurements of oxygen consumption were performed using the high-resolution Oroboros Oxygraph-2k (Oroboros, Innsbruck, Austria). Two substrate-uncoupler-inhibitor-titration protocols were used in this study (i) to measure maximal capacity of oxidative phosphorylation and (ii) to identify specific flux control ratios suggestive of mitochondrial efficiency or coupling capacity (respiratory control ratio and leak control coupling). Analysis of citrate synthase activity is also reported in our previous report.[31]

Statistics

Nonparametric Mann-Whitney U-test was used to test the effect of LHTL (versus placebo). Within a given group (placebo or LHTL), the effect of time or isovolumic haemodilution (baseline, W4, W4 after isovolumic haemodilution) on the different parameters was evaluated with the use of Friedman

non-parametric test and planned pair-wise-specific comparisons were made using the Wilcoxon test. The same procedure was also applied by considering three groups rather than two (ie, placebo (n=6); LHTL$^{(+)}$ (n=5) and LHTL$^{(-)}$ (n=5)), LHTL$^{(+)}$ and LHTL$^{(-)}$, respectively, referring to the subgroups of 'responders' and 'non-responders', that is, the athletes who did (or did not) increase their Hb_{mass} following 4 weeks of LHTL (W4). Statistics were carried out with the Statview 5.0 software (SAS Institute, Cary, North Carolina, USA). The values are reported as arithmetic means±SD unless otherwise stated. Differences were considered as significant for $p<0.05$.

RESULTS

Four weeks of normobaric LHTL did not increase VO_2max

LHTL resulted in a non-significant increase in VO_2max (relative to body mass), being 1.5% in LHTL (n=10) and 2.0% in placebo subjects (n=6) (table 1). Maximal workload was non-significantly augmented by 2.2% in LHTL and by 2.7% in placebo subjects.[26]

LHTL induced an increase in Hb_{mass} in 50% of the athletes

The increase in Hb_{mass} among the 10 athletes exposed to LHTL was marginal since five subjects did not increase Hb_{mass} (LHTL$^{(-)}$), while the other five experienced a 4.6% increase in Hb_{mass} (LHTL$^{(+)}$) (table 1 and figure 1). All subjects developed haemoconcentration during the study.[26] Blood viscosity was similar between groups, and unchanged after LHTL or placebo intervention (W4).

LHTL-induced gain in Hb_{mass} is not associated with a concurrent increase in VO_2max

Maximal workload increased in LHTL$^{(+)}$ but not in LHTL$^{(-)}$, suggesting a link between Hb_{mass} and performance gain after LHTL (table 1); however this was not supported by VO_2max data, indicating that LHTL$^{(+)}$ athletes experienced a non-significant 1.0% gain in VO_2max after LHTL, comparable to the also non-significant 2.1% gain observed among LHTL$^{(-)}$ athletes (table 1 and figure 1).

Suppression of the LHTL-induced gain in Hb_{mass} resulted in a significant reduction of VO_2max

LHTL$^{(+)}$ subjects presenting with Hb_{mass} expansion and plasma volume contraction after LHTL underwent blood withdrawal (280±74 ml), followed by plasma expander infusion (540±89 ml), while LHTL$^{(-)}$ and placebo subjects, presenting with no change in Hb_{mass} but plasma volume contraction, received plasma expander only, respectively, 572±299 and 567 ±169 ml. These interventions allowed for [Hb] to decrease, on average (n=16), from 15.0±0.8 g/dl before isovolumic haemodilution to 14.1±0.7 g/dl after isovolumic haemodilution, that is, at a level comparable to baseline value (14.0±0.5 g/dl).

Isovolumic haemodilution decreased VO_2max in LHTL$^{(+)}$, while plasma expander infusion per se had no effect on VO_2max in LHTL$^{(-)}$ (table 1 and figure 1). Maximal workload was reduced in LHTL$^{(+)}$ but not in LHTL$^{(-)}$. Plasma expander infusion did not significantly alter maximal workload in placebo athletes but did decrease their VO_2max significantly.

Cycling efficiency was not altered following LHTL

Cycling efficiency at 150 and 200 W remained unchanged throughout the study, in both the placebo and LHTL groups.[26] Isovolumic haemodilution did not alter cycling efficiency (table 1).

LHTL was not associated with changes in mitochondrial content or function

Mitochondrial content (ie, citrate synthase activity) was altered neither by LHTL nor by time (figure 2A). Mitochondrial function (oxygen flux per unit of mitochondrial content) was similar in both groups, both at baseline and at W4 (figure 2B). The respiratory control ratio and leak control coupling, both indicative of mitochondrial efficiency, were found similar

Table 1 Submaximal exercise, maximal exercise and total haemoglobin mass

	Placebo (n=6)		LHTL$^{(+)}$ (n=5)		LHTL$^{(-)}$ (n=5)	
	Baseline	W4	Baseline	W4	Baseline	W4
Submaximal O_2 uptake						
VO_2 at 150 W (l/min)	2472±288	2447±170	2438±160	2351±83	2409±147	2366±186
Isovolumic haemodilution	–	2396±212	–	2392±167	–	2332±218
VO_2 at 200 W (l/min)	3007±212†	3015±148†	3043±158	2957±177	3097±106	3005±91
Isovolumic haemodilution	–	2998±313	–	2937±165	–	3022±138
Maximal O_2 uptake and workload						
VO_2max (ml/min)	4948±446†	5050±485†	4924±582	4958±650	4557±726	4521±676
Isovolumic haemodilution	–	4797±504	–	4672±583	–	4536±722
VO_2max (ml/min/kg)	70.8±5.3	72.3±6.0	73.5±2.9	74.3±4.8	65.1±4.8	66.5±5.5
Isovolumic haemodilution	–	68.6±5.2**	–	70.0±4.0**	–	66.6±4.6
Maximal workload (W)	411±23†	422±28†	412±50	432±49*	398±72	394±62
Isovolumic haemodilution	–	412±29	–	406±48**	–	386±61
Total haemoglobin mass						
Hb_{mass} (g)	984±98†	971±80†	928±92	970±93*	933±219	908±225
Hb_{mass} (g/kg)	14.06±0.76	13.88±0.60	13.87±0.97	14.58±0.95*	13.14±1.00	13.12±1.15

Values are means±SD. Within the live high–train low (LHTL) group of 10 athletes, LHTL$^{(+)}$ and LHTL$^{(-)}$, respectively, refer to the subgroups of 'responders' and 'non-responders', that is, the athletes who did (or did not) increase their total haemoglobin mass (Hb_{mass}) following 4 weeks of LHTL (W4). Isovolumic haemodilution was performed at W4, after the first incremental test had been completed. LHTL$^{(+)}$ subjects were isovolumically haemodiluted by means of phlebotomy and plasma volume expander (PVX) infusion, whereas LHTL$^{(-)}$ and placebo subjects received PVX infusion only. Hb_{mass}, total haemoglobin mass (absolute and adjusted to body weight); VO_2max, maximal O_2 uptake (absolute and adjusted to body weight); maximal workload (W_{max}) was calculated as: $W_{max}=W_{compl}+25\times(t/60)$, where W_{compl} is the last workload completed, t is seconds that the final, not completed workload was sustained and 25 (W) is the increment in workload. For VO_2 at 200 W, n=4 for LHTL$^{(-)}$.

*$p<0.05$ vs baseline, **$p<0.05$ vs W4.

†These data are reproduced from our previous report[26] for the sake of clarity.

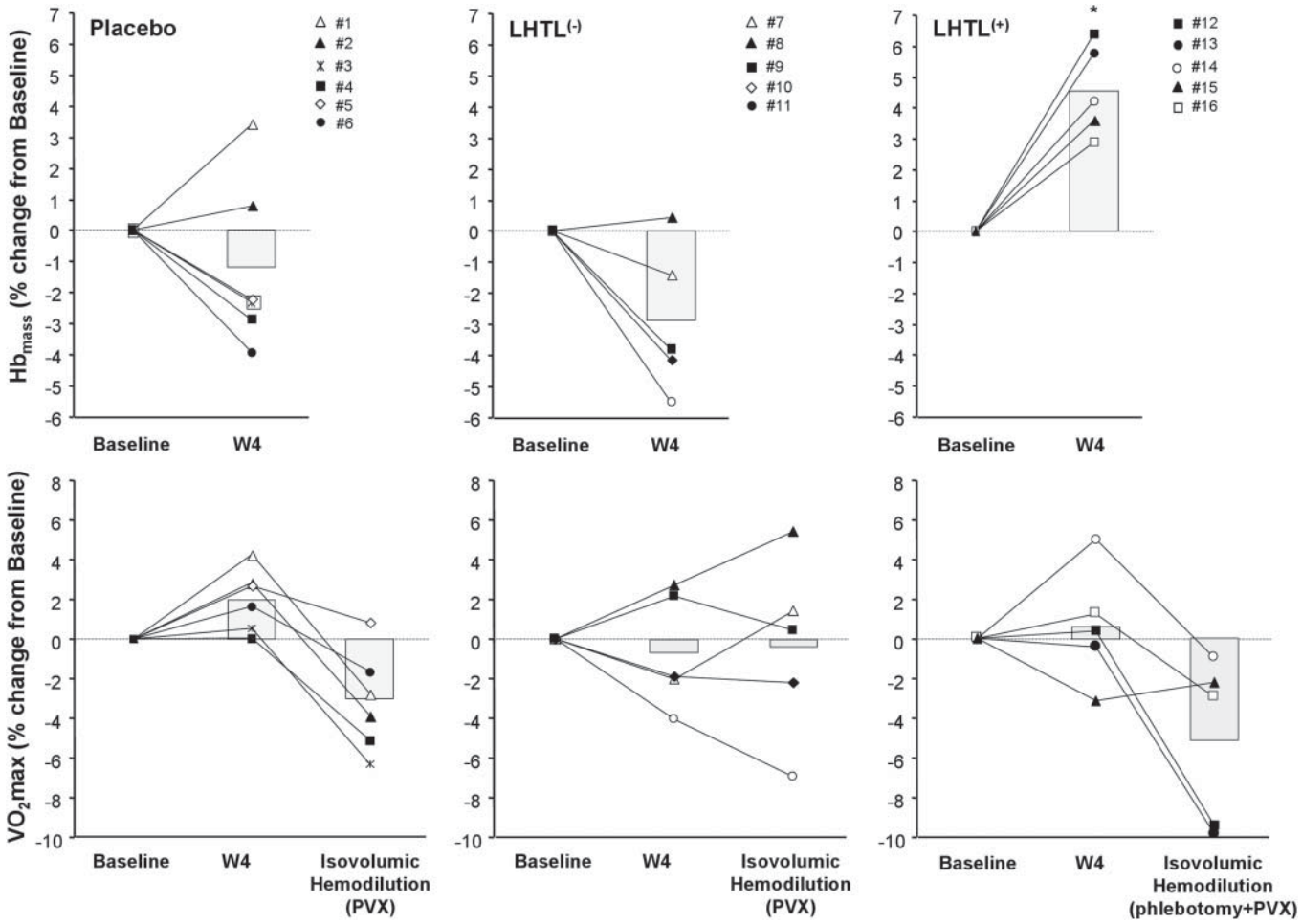

Figure 1 The effects of live high–train low (LHTL) and isovolumic haemodilution on haemoglobin mass (Hb_{mass}) and VO_2max. The upper panels show the individual response of total Hb_{mass} (in absolute values), induced by 4 weeks of placebo (n=6) or live high—train low intervention (LHTL; n=10). LHTL athletes were divided, according to their individual changes in Hb_{mass}, into 'responders' ($LHTL^{(+)}$, n=5) or 'non-responders' ($LHTL^{(-)}$, n=5). Of note is that intervention was associated with a decrease in plasma volume in all subjects.[26] The lower panels show first the concurrent individual changes in VO_2max (in absolute values), in response to LHTL or placebo intervention (W4 vs baseline), and second the modulation of these changes in response to acute isovolumic haemodilution, aimed at restoring intravascular volumes to basal values. The subjects who gained Hb_{mass} after intervention (ie, $LHTL^{(+)}$) were isovolumically haemodiluted by means of phlebotomy and plasma volume expander (PVX) infusion, whereas those who did not increase their Hb_{mass} (ie, $LHTL^{(-)}$ and placebo) received PVX infusion only. Histograms indicate means. *p<0.05 vs baseline; **p<0.05 vs W4.

between groups at baseline and unchanged following LHTL or placebo intervention (figure 2C,D).

DISCUSSION

Using a double-blinded, placebo-controlled design we found that LHTL induced no positive effect on VO_2max in endurance athletes. Only 5 of 10 LHTL athletes responded by increasing Hb_{mass}. These 'responders' also increased maximal workload but not VO_2max in comparison with either their non-responding LHTL counterparts or the placebo athletes. The importance of Hb_{mass} and/or [Hb] for O_2 transport during maximal exercise was however confirmed since isovolumic haemodilution resulted in a decrease in both VO_2max and maximal workload. Finally, LHTL did not affect skeletal muscle mitochondrial function or whole body cycling efficiency.

Response of Hb_{mass} to LHTL and its role on VO_2max

Data analysis revealed that 5 of 10 LHTL subjects did increase Hb_{mass}, suggesting a significant variability among athletes with regard to the haematological response to hypoxia, and that moderate hypoxia may provide, at least in some athletes, a sufficient stimulus for red blood cell synthesis to be increased. Similarly, Chapman *et al*[19] found that only 53% (17 of 32 subjects) exhibited an expansion of RCV in response to altitude training. The different Hb_{mass} response among our LHTL subjects was not explained by the degree of haemoconcentration or by the increase of reticulocyte count. Also an increase in Hb_{mass} following LHTL was not accompanied by a concomitant rise in VO_2max, confirming previous data,[9] which questions the significance of the small Hb_{mass} improvements detected in athletes undergoing the LHTL regimen. Of note, after blood transfusion, only 50% of the added O_2 delivery may be utilised by the tissues.[32] Nevertheless, it could be that highly trained individuals require a larger increase in Hb_{mass} to increase VO_2max as normal healthy individuals.

In spite of their 'insensitivity' to slightly increased O_2 delivery, the LHTL athletes were critically dependent on convective O_2 transport, since acute normalisation of Hb_{mass} via isovolumic haemodilution provoked a significant drop in maximal power output and VO_2max. Similarly, lowering of Hb_{mass} through

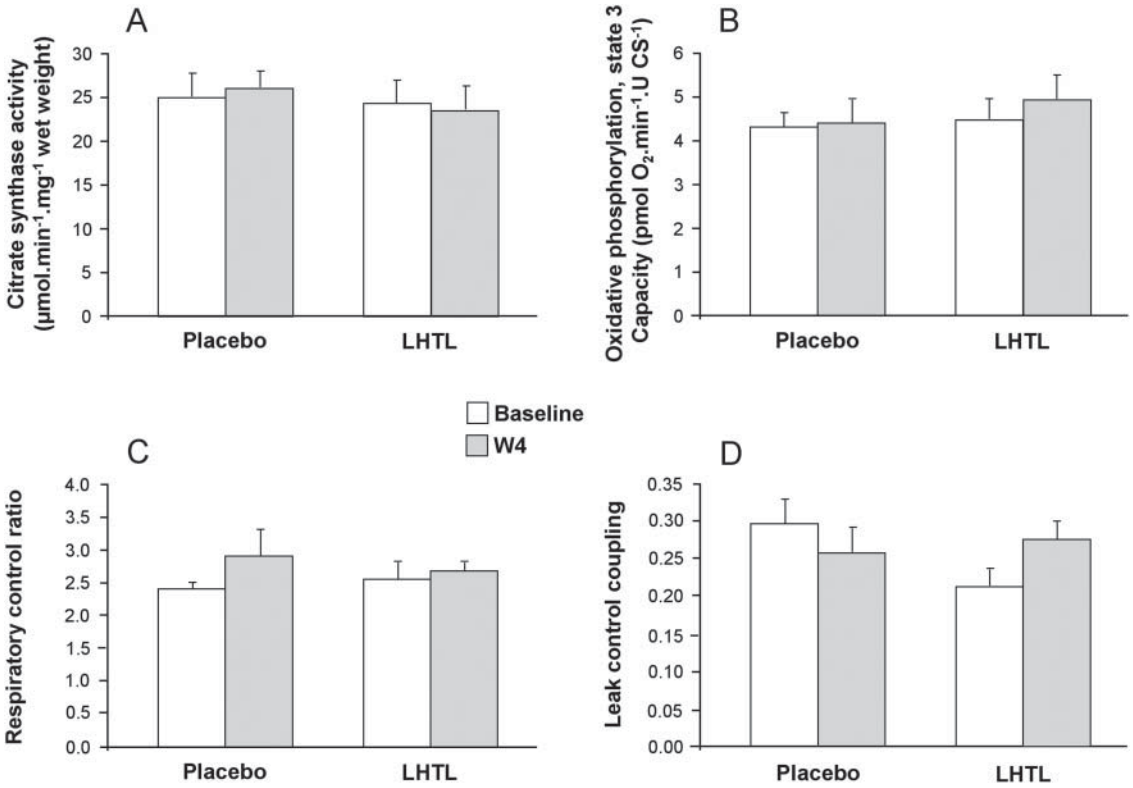

Figure 2 Live high–train low (LHTL) does not alter mitochondrial function of skeletal muscle. The figure shows the mitochondrial function of skeletal muscle in subjects assigned to the placebo (n=6) and LHTL (n=9) groups. Open bars represent baseline measurements and grey bars represent measurements following 4 weeks of respective treatment (W4). Panel A shows citrate synthase activity. Panel B shows maximal oxidative phosphorylation capacity, expressed per unit of citrate synthase activity. Panel C shows respiratory control ratio, presented as the ratio of maximal state 3 respiration through complex I to malate-induced (2 mM) and pyruvate-induced (5 mM) leak respiration. Panel D shows leak coupling control presented as the ratio of malate-induced (2 mM) and pyruvate-induced (5 mM) leak respiration to maximal state 3 respiration through complex I (PI). Values are reported as mean±SE.

blood donation[33] or haemodilution[16] decreases VO_2max. On the contrary, the effect of clamping Hb_{mass} during LHTL (via repeated phlebotomy) on maximal aerobic performance was found to be marginal,[10] but such procedure substantially differs from acute isovolumic haemodilution.

An important factor to consider during blood withdrawal and/or haemodilution (secondary to plasma expander infusion) is the acute reduction in [Hb], which is known to decrease VO_2max, because of a reduction of the oxygen-carrying capacity of blood.[34] Although not in line with previous data in athletes indicating no change in VO_2max following plasma volume expansion,[35] our finding demonstrating that VO_2max decreased after haemodilution in the placebo group confirms the important role of [Hb] on VO_2max, further suggesting that Hb_{mass} per se is not the only factor controlling VO_2max after isovolumic haemodilution in the $LHTL^{(+)}$ group. Conversely, the finding that haemodilution did not affect VO_2max in the $LHTL^{(-)}$ group suggests only a minor contribution of [Hb]. Whatever the exact role of [Hb] on VO_2max, our data imply that $LHTL^{(-)}$ athletes, unlike placebo subjects, compensated for reduced arterial O_2 content by achieving higher maximal cardiac output and/or O_2 extraction. Although stroke volume was not measured in the present study, we speculate that cardiac response after isovolumic haemodilution was not much different between placebo and LHTL subjects since (i) infused volume was identical and (ii) decreases in maximal heart rate were small (3–4 beats/min) and similar between groups.

We cannot exclude that incomplete recovery after the first VO_2max test may have contributed to some fatigue during the second exercise bout. However, VO_2max should have recovered within 2 h[36 37] and athletes are accustomed to perform repeated bouts of high-intensity exercise.

Mitochondrial function is not improved following LHTL

Hypoxic regulation of mitochondrial expression is not well understood. Skeletal muscle mitochondrial volume density,[38] enzyme activities[39] and protein content[40] have all been observed to decrease following extended hypoxic exposure while other studies have shown negligible differences in mitochondrial-specific biochemical expression.[41–44] We have previously found no significant alteration in respiratory chain function in lowland natives measured at sea level and again following 7–9 days of high-altitude exposure,[45] and the findings in the current study support these data as mitochondrial function in the LHTL subjects did not differ from data obtained in the control subjects. The present observation at the tissue level provides a basis for explaining why cycling efficiency remained unchanged following LHTL. Other explanations may be related to unchanged motor control and/or biomechanics, which are also determinants of exercise efficiency.[46]

CONCLUSION

In the present study VO_2max did not increase in response to LHTL and potential mechanisms that could influence VO_2max were not identified: we evaluated both central and peripheral adaptations to LHTL and found no effect in highly trained individuals. Furthermore, even though LHTL caused modest erythrocyte volume expansion in some individuals, these

subjects did not enhance their VO_2max. In conclusion, the positive effects of LHTL on O_2 transport appear to be negligible among elite cyclists who already possess very high aerobic capacities conferred by high Hb_{mass} and VO_2max.

What this study adds

- This study provides a mechanistic basis to explain why normobaric live high–train low (LHTL) does not boost VO_2max in endurance-trained athletes:
  - The modest increase in haemoglobin mass conferred by LHTL is not sufficient to further increase the athlete's high VO_2max, even if VO_2max critically depends on Hb_{mass} in this population.
  - Unchanged maximal oxidative capacity or mitochondrial efficiency after LHTL provides evidence against the hypothesis that LHTL may improve muscle efficiency and therefore VO_2max.
- Extrapolation to natural altitude is limited since hypobaric hypoxia could induce different responses than those occurring with normobaric hypoxia,[47 48] although this seems unlikely to have major implications in the present setting.

Acknowledgements We are grateful to the participants in the study. The authors would like to acknowledge Oroboros Instruments (Innsbruck, Austria) for making valuable equipment at our disposal during the study period. We also would like to thank Ms. Anne Schaerrer and her team (Le Sentier hospital) for excellent logistical support throughout the experiment, as well as the CNSN team (Prémanon) for valuable help during the study.

Contributors PRo, CS, NC, MM and CL designed the research; PRo, CS, RAJ, PRa, NN, DP, VD, AC, JF, NHS and AP collected data; PRo, CS, RAJ, P Ra, NN, DP, EG, VD and AP analysed data; PRo, CS, RAJ, EG and CL interpreted results of experiments; PRo, CS, RAJ, PRa and CL wrote the manuscript.

Funding This study was funded through grants obtained from the Bundes Amt für Sport (BASPO, Switzerland), Team Danmark (Denmark) and Ministère des Sports/ Institut National du Sport, de l'Expertise et de la Performance (France).

Ethics approval Ethics Committee of Zurich (2010-066/0) and Vaud (215/10) (Switzerland).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Levine BD,** Stray-Gundersen J. A practical approach to altitude training: where to live and train for optimal performance enhancement. *Int J Sports Med* 1992;**13** (Suppl 1):S209–12.
2. **Levine BD,** Stray-Gundersen J. 'Living high-training low': effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 1997;**83**:102–12.
3. **Stray-Gundersen J,** Chapman RF, Levine BD. 'Living high-training low' altitude training improves sea level performance in male and female elite runners. *J Appl Physiol* 2001;**91**:1113–20.
4. **Levine BD,** Stray-Gundersen J. Point: positive effects of intermittent hypoxia (live high: train low) on exercise performance are mediated primarily by augmented red cell volume. *J Appl Physiol* 2005;**99**:2053–5.
5. **Gore CJ,** Hopkins WG. Counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume. *J Appl Physiol* 2005;**99**:2055–7; discussion 2057–8.
6. **Bonetti DL,** Hopkins WG. Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med* 2009;**39**:107–27.
7. **Gore CJ,** Clark SA, Saunders PU. Nonhematological mechanisms of improved sea-level performance after hypoxic exposure. *Med Sci Sports Exerc* 2007;**39**:1600–9.
8. **Weil JV,** Jamieson G, Brown DW, *et al*. The red cell mass—arterial oxygen relationship in normal man. Application to patients with chronic obstructive airway disease. *J Clin Invest* 1968;**47**:1627–39.
9. **Clark SA,** Quod MJ, Clark MA, *et al*. Time course of haemoglobin mass during 21 days live high: train low simulated altitude. *Eur J Appl Physiol* 2009;**106**:399–406.
10. **Garvican LA,** Pottgiesser T, Martin DT, *et al*. The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. *Eur J Appl Physiol* 2011;**111**:1089–101.
11. **Brugniaux JV,** Schmitt L, Robach P, *et al*. Eighteen days of 'living high, training low' stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl Physiol* 2006;**100**:203–11.
12. **Robach P,** Schmitt L, Brugniaux JV, *et al*. Living high-training low: effect on erythropoiesis and aerobic performance in highly-trained swimmers. *Eur J Appl Physiol* 2006;**96**:423–33.
13. **Robertson EY,** Saunders PU, Pyne DB, *et al*. Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc* 2010;**42**:394–401.
14. **Wehrlin JP,** Zuest P, Hallen J, *et al*. Live high-train low for 24 days increases hemoglobin mass and red cell volume in elite endurance athletes. *J Appl Physiol* 2006;**100**:1938–45.
15. **Saunders PU,** Ahlgrim C, Vallance B, *et al*. An attempt to quantify the placebo effect from a three-week simulated altitude training camp in elite race walkers. *Int J Sports Physiol Perform* 2010;**5**:521–34.
16. **Kanstrup IL,** Ekblom B. Blood volume and hemoglobin concentration as determinants of maximal aerobic power. *Med Sci Sports Exerc* 1984;**16**:256–62.
17. **Ekblom B,** Berglund B. Effect of erythropoietin administration on maximal aerobic power. *Scand J Med Sci Sports* 1991;**1**:88–93.
18. **Lundby C,** Robach P, Boushel R, *et al*. Does recombinant human Epo increase exercise capacity by means other than augmenting oxygen transport? *J Appl Physiol* 2008;**105**:581–7.
19. **Chapman RF,** Stray-Gundersen J, Levine BD. Individual variation in response to altitude training. *J Appl Physiol* 1998;**85**:1448–56.
20. **Neya M,** Enoki T, Kumai Y, *et al*. The effects of nightly normobaric hypoxia and high intensity training under intermittent normobaric hypoxia on running economy and hemoglobin mass. *J Appl Physiol* 2007;**103**:828–34.
21. **Saunders PU,** Telford RD, Pyne DB, *et al*. Improved running economy in elite runners after 20 days of simulated moderate-altitude exposure. *J Appl Physiol* 2004;**96**:931–7.
22. **Gore CJ,** Hahn AG, Aughey RJ, *et al*. Live high: train low increases muscle buffer capacity and submaximal cycling efficiency. *Acta Physiol Scand* 2001;**173**:275–86.
23. **Ponsot E,** Dufour SP, Zoll J, *et al*. Exercise training in normobaric hypoxia in endurance runners. II. Improvement of mitochondrial properties in skeletal muscle. *J Appl Physiol* 2006;**100**:1249–57.
24. **Mizuno M,** Savard GK, Areskog NH, *et al*. Skeletal muscle adaptations to prolonged exposure to extreme altitude: a role of physical activity? *High Alt Med Biol* 2008;**9**:311–17.
25. **Lundby C,** Calbet JA, Sander M, *et al*. Exercise economy does not change after acclimatization to moderate to very high altitude. *Scand J Med Sci Sports* 2007;**17**:281–91.
26. **Siebenmann C,** Robach P, Jacobs RA, *et al*. 'Live high—train low' using normobaric hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol* 2012;**112**:106–17.
27. **Nordsborg NB,** Siebenmann C, Jacobs RA, *et al*. Four weeks of normobaric 'Live High—Train Low' does not alter muscular or systemic capacity for maintaining pH and K+ homeostasis during intense exercise. *J Appl Physiol* 2012;**112**:2027–36.
28. **American Thoracic Society, American College of Chest Physicians**. ATS/ACCP statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2003;**167**:211–77.
29. **Lundby C,** Thomsen JJ, Boushel R, *et al*. Erythropoietin treatment elevates haemoglobin concentration by increasing red cell volume and depressing plasma volume. *J Physiol* 2007;**578**:309–14.
30. **Burge CM,** Skinner SL. Determination of hemoglobin mass and blood volume with CO: evaluation and application of a method. *J Appl Physiol* 1995;**79**:623–31.
31. **Jacobs RA,** Rasmussen P, Siebenmann C, *et al*. Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol* 2011;**111**:1422–30.
32. **Turner DL,** Hoppeler H, Noti C, *et al*. Limitations to VO_2max in humans after blood retransfusion. *Respir Physiol* 1993;**92**:329–41.
33. **Burnley M,** Roberts CL, Thatcher R, *et al*. Influence of blood donation on O_2 uptake on-kinetics, peak O_2 uptake and time to exhaustion during severe-intensity cycle exercise in humans. *Exp Physiol* 2006;**91**:499–509.
34. **Calbet JA,** Lundby C, Koskolou M, *et al*. Importance of hemoglobin concentration to exercise: acute manipulations. *Respir Physiol Neurobiol* 2006;**151**:132–40.
35. **Warburton DE,** Gledhill N, Jamnik VK, *et al*. Induced hypervolemia, cardiac function, VO_2max, and performance of elite cyclists. *Med Sci Sports Exerc* 1999;**31**:800–8.
36. **McKenzie DC,** Lama IL, Potts JE, *et al*. The effect of repeat exercise on pulmonary diffusing capacity and EIH in trained athletes. *Med Sci Sports Exerc* 1999;**31**:99–104.
37. **Caillaud CF,** Anselme FM, Prefaut CG. Effects of two successive maximal exercise tests on pulmonary gas exchange in athletes. *Eur J Appl Physiol Occup Physiol* 1996;**74**:141–7.
38. **Hoppeler H,** Kleinert E, Schlegel C, *et al*. Morphological adaptations of human skeletal muscle to chronic hypoxia. *Int J Sports Med* 1990;**11**(Suppl 1):S3–9.

39. **Howald H,** Pette D, Simoneau JA, *et al*. Effect of chronic hypoxia on muscle enzyme activities. *Int J Sports Med* 1990;**11**(Suppl 1):S10–14.
40. **Vigano A,** Ripamonti M, De Palma S, *et al*. Proteins modulation in human skeletal muscle in the early phase of adaptation to hypobaric hypoxia. *Proteomics* 2008;**8**:4668–79.
41. **Green H,** Roy B, Grant S, *et al*. Downregulation in muscle Na(+)-K(+)-ATPase following a 21-day expedition to 6194 m. *J Appl Physiol* 2000;**88**:634–40.
42. **Green HJ,** Sutton JR, Cymerman A, *et al*. Operation Everest II: adaptations in human skeletal muscle. *J Appl Physiol* 1989;**66**:2454–61.
43. **Green HJ,** Sutton JR, Wolfel EE, *et al*. Altitude acclimatization and energy metabolic adaptations in skeletal muscle during exercise. *J Appl Physiol* 1992;**73**:2701–8.
44. **Young AJ,** Evans WJ, Fisher EC, *et al*. Skeletal muscle metabolism of sea-level natives following short-term high-altitude residence. *Eur J Appl Physiol Occup Physiol* 1984;**52**:463–6.
45. **Jacobs RA,** Boushel R, Wright-Paradis C, *et al*. Mitochondrial function in human skeletal muscle following high altitude exposure. *Exp Physiol* 2012 DOI:10.1113/expphysiol.2012.06.6092.
46. **Saunders PU,** Pyne DB, Telford RD, *et al*. Factors affecting running economy in trained distance runners. *Sports Med* 2004;**34**:465–85.
47. **Millet GP,** Faiss R, Pialoux V. Point: counterpoint: hypobaric hypoxia induces/does not induce different responses from normobaric hypoxia. *J Appl Physiol* 2012;**112**:1783–4.
48. **Mounier R,** Brugniaux JV. Counterpoint: hypobaric hypoxia does not induce different responses from normobaric hypoxia. *J Appl Physiol* 2012;**112**:1784–6.

3. Discussion and outlook

The aim of this project was to enhance our understanding of the interaction between hypoxia and aerobic exercise. Specifically, we tested whether the HPV-induced rise in PAP and the decline in cerebral oxygenation contribute to the limited exercise capacity in acute hypoxia. Furthermore, we examined the effect of LHTL altitude training on the performance of elite athletes and evaluated underlying physiological mechanisms. As the results obtained in each study are discussed in detail in the corresponding articles, this section summarizes the key points and highlights future directions for research within the fields.

3.1 Limitations to exercise in acute hypoxia induced by the pulmonary circulation

The reduced VO_2max in acute hypoxia is mainly related to an attenuated skeletal muscle O_2 supply, which is induced by the detrimental effect of hypoxia on c_aO_2 and, in severe hypoxia, maximal cardiac output (25, 125). It is notable that these mechanisms also constitute the main reasons for exercise intolerance in pulmonary hypertension patients (89, 90, 124). As the HPV transiently induces pulmonary hypertension (87) this may thus contribute to the factors that limit VO_2max in hypoxia. We tested this hypothesis in HAPE-susceptible individuals as they experience an excessive HPV and are thus convenient subjects for this purpose.

In agreement with an earlier study (40) our results indicate that pharmacological attenuation of PAP partially restores hypoxic VO_2max in HAPE-susceptible subjects without affecting SaO_2 during maximal exercise (114). Subsequently, the direct comparison between normal and HAPE-susceptible subjects revealed a higher PAP during exercise in hypoxia and a tendency for a more pronounced decrease in VO_2max in the latter but no differences in SaO_2 at exhaustion (115). Taken together these findings support the concept of PAP as a limiting factor for exercise in hypoxia and favour right ventricular restriction rather than an impaired pulmonary O_2 diffusion as perpetrator. This mechanism might at least partly underlie the largely unexplained reduction in maximal cardiac output that contributes to the decline in VO_2max at altitudes > 4,500 m (25). Nevertheless, as it was the case in previous studies (38-40, 88, 97) we could not include measurements of maximal cardiac output and direct evidence for this hypothesis thus remains to be provided.

Accordingly, the aim of our next study within this area is a direct assessment of the impact of PAP on the reduction in maximal cardiac output in severe hypoxia. The key intervention will be the catheterization of the pulmonary artery which allows for reliable measurement of PAP and cardiac output also during maximal exercise (120, 130). Shortly before exhaustion acute

specific pulmonary vasodilation will be induced by inspiratory nitric oxide (NO) administration (43) and the resulting effect on maximal cardiac output and VO_2max will be determined.

3.2 Limitations to exercise in acute hypoxia induced by the cerebral circulation

Hypoxia promotes the development of central fatigue by increasing the discharge frequency of inhibitory muscle afferents (60, 72). Furthermore, observations during acute re-oxygenation have led to speculations that hypoxia may accelerate the onset of central fatigue by attenuation of cerebral oxygenation (8, 10).

The second aim of this project was to provide direct evidence for this mechanism. By means of inspiratory CO_2 supplementation we restored oxygenation specifically in the brain while the remaining organism was kept hypoxic (117). However, contrary to our hypothesis, and in line with the previous observations in more severe hypoxia (123), this did not increase VO_2max and thus argues against cerebral hypoxia as a limiting factor for maximal exercise in acute hypoxia. Nevertheless, this does not rule out a limiting role in other types of exercise, as the incremental VO_2max test induces a progressive demand for muscular O_2 supply which may overcharge the cardiovascular system and lead to exhaustion before cerebral hypoxia becomes critical (17, 68, 109, 110). Future studies could thus examine whether inspiratory CO_2 administration benefits performance in submaximal exercise tests in hypoxia. In addition, simultaneous infusion of bicarbonate may be applied to prevent the CO_2 administration from accelerating the development of fatiguing acidosis (108) which may have biased the results of present and past work (123).

3.3 The effect of Live High-Train Low on the exercise capacity of elite athletes

Numerous studies in the past have indicated that acclimatization to hypoxia partially restores aerobic exercise capacity (62, 82, 113). As the underlying physiological adaptations may also improve performance in normoxia the use of preliminary hypoxic exposure has become a popular measure in the preparation for sea level events (76). Over the last four decades different altitude training strategies have been developed (111) among which LHTL appears to be the most promising (69). Nevertheless, there is disagreement regarding the efficiency of this training form, and the mechanisms underlying potential performance gains, as divergent results have been reported (112). Furthermore, the relevance of most positive findings are

limited by suboptimal study designs and the inclusion of inappropriate subjects (76). The last aim of this project was thus to test the effects of LHTL on elite athletes in a double-blinded and placebo-controlled trial and to determine the mechanisms underlying potential performance gains.

Contrary to our hypothesis, the conducted study revealed that despite extensive hypoxic exposure no haematological (116) or skeletal muscle adaptations (102) were induced and, as a result, both VO_2max and time trial performance remained unaffected by LHTL. Besides nourishing the speculations that previous findings may have been related to a placebo-effect (117) this led us to the conclusion that either a high baseline RCV made the elite athletes insensitive to the natural rise in circulating erythropoietin (76) or that four weeks of discontinuous exposure to moderate hypoxia are simply insufficient to stimulate erythropoiesis.

In the near future we plan to conduct studies that clarify this issue. In a first attempt we aim to assess whether a four week sojourn at the Jungfraujoch research station (3,454 m) stimulates erythropoiesis in normally trained individuals. If an increase in RCV will be observed under these conditions the next step will be to conduct another LHTL study including both, elite athletes and normal subjects and compare the responses of these two populations. In contrast, if even a continuous residence at an altitude above that normally applied in LHTL will fail to stimulate erythropoiesis in non-athletes we will discard the commonly used LHTL strategy as a useful training tool. In this case it might be an option to assess whether an ergogenic adaptation may be induced if the degree of hypoxia is increased above the conventionally applied as it was hypothesized previously (100). Athletes have traditionally refrained from such a modification as they feared negative consequences for recovery or immunofunction (129). However, to date, this lacks solid evidence and could thus be another issue to clarify in a future LHTL study applying more severe hypoxia.

4. Bibliography

1. Abbrecht PH and Littell JK. Plasma erythropoietin in men and mice during acclimatization to different altitudes. *J Appl Physiol* 32: 54-58, 1972.
2. Adams WC, Bernauer EM, Dill DB, and Bomar JB, Jr. Effects of equivalent sea-level and altitude training on VO2max and running performance. *J Appl Physiol* 39: 262-266, 1975.
3. Allen DG. Fatigue in working muscles. *J Appl Physiol* 106: 358-359, 2009.
4. Allen DG, Lamb GD, and Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88: 287-332, 2008.
5. Amann M. Central and peripheral fatigue: interaction during cycling exercise in humans. *Med Sci Sports Exerc* 43: 2039-2045, 2011.
6. Amann M. Significance of group III and IV muscle afferents for the endurance exercising human. *Clin Exp Pharmacol Physiol*, 2012.
7. Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, and Dempsey JA. Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *J Physiol* 589: 5299-5309, 2011.
8. Amann M and Calbet JA. Convective oxygen transport and fatigue. *J Appl Physiol* 104: 861-870, 2008.
9. Amann M and Dempsey JA. Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *J Physiol* 586: 161-173, 2008.
10. Amann M and Kayser B. Nervous system function during exercise in hypoxia. *High Alt Med Biol* 10: 149-164, 2009.
11. Amann M, Proctor LT, Sebranek JJ, Eldridge MW, Pegelow DF, and Dempsey JA. Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *J Appl Physiol* 105: 1714-1724, 2008.
12. Amann M, Romer LM, Subudhi AW, Pegelow DF, and Dempsey JA. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J Physiol* 581: 389-403, 2007.
13. Astrand PO and Saltin B. Oxygen uptake during the first minutes of heavy muscular exercise. *J Appl Physiol* 16: 971-976, 1961.
14. Babault N, Desbrosses K, Fabre MS, Michaut A, and Pousson M. Neuromuscular fatigue development during maximal concentric and isometric knee extensions. *J Appl Physiol* 100: 780-785, 2006.
15. Bartsch P, Mairbaurl H, Maggiorini M, and Swenson ER. Physiological aspects of high-altitude pulmonary edema. *J Appl Physiol* 98: 1101-1110, 2005.
16. Bartsch P, Mairbaurl H, Swenson ER, and Maggiorini M. High altitude pulmonary oedema. *Swiss Med Wkly* 133: 377-384, 2003.
17. Bassett DR, Jr. and Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 32: 70-84, 2000.

18. Bender PR, Groves BM, McCullough RE, McCullough RG, Huang SY, Hamilton AJ, Wagner PD, Cymerman A, and Reeves JT. Oxygen transport to exercising leg in chronic hypoxia. *J Appl Physiol* 65: 2592-2597, 1988.

19. Bonetti DL and Hopkins WG. Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med* 39: 107-127, 2009.

20. Boushel R, Gnaiger E, Calbet JA, Gonzalez-Alonso J, Wright-Paradis C, Sondergaard H, Ara I, Helge JW, and Saltin B. Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion* 11: 303-307, 2011.

21. Brugniaux JV, Schmitt L, Robach P, Nicolet G, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Saas P, Chorvot MC, Cornolo J, Olsen NV, and Richalet JP. Eighteen days of "living high, training low" stimulate erythropoiesis and enhance aerobic performance in elite middle-distance runners. *J Appl Physiol* 100: 203-211, 2006.

22. Buick FJ, Gledhill N, Froese AB, Spriet L, and Meyers EC. Effect of induced erythrocythemia on aerobic work capacity. *J Appl Physiol* 48: 636-642, 1980.

23. Burtscher M, Nachbauer W, Baumgartl P, and Philadelphy M. Benefits of training at moderate altitude versus sea level training in amateur runners. *Eur J Appl Physiol Occup Physiol* 74: 558-563, 1996.

24. Calbet JA. The rate of fatigue accumulation as a sensed variable. *J Physiol* 575: 688-689, 2006.

25. Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD, and Saltin B. Determinants of maximal oxygen uptake in severe acute hypoxia. *Am J Physiol Regul Integr Comp Physiol* 284: R291-303, 2003.

26. Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD, and Saltin B. Why is VO2 max after altitude acclimatization still reduced despite normalization of arterial O2 content? *Am J Physiol Regul Integr Comp Physiol* 284: R304-316, 2003.

27. Calbet JA and Lundby C. Air to muscle O2 delivery during exercise at altitude. *High Alt Med Biol* 10: 123-134, 2009.

28. Cerretelli P. Limiting factors to oxygen transport on Mount Everest. *J Appl Physiol* 40: 658-667, 1976.

29. Clark VR, Hopkins WG, Hawley JA, and Burke LM. Placebo effect of carbohydrate feedings during a 40-km cycling time trial. *Med Sci Sports Exerc* 32: 1642-1647, 2000.

30. Cymerman A, Reeves JT, Sutton JR, Rock PB, Groves BM, Malconian MK, Young PM, Wagner PD, and Houston CS. Operation Everest II: maximal oxygen uptake at extreme altitude. *J Appl Physiol* 66: 2446-2453, 1989.

31. D'Alonzo GE, Gianotti LA, Pohil RL, Reagle RR, DuRee SL, Fuentes F, and Dantzker DR. Comparison of progressive exercise performance of normal subjects and patients with primary pulmonary hypertension. *Chest* 92: 57-62, 1987.

32. Decorte N, Lafaix PA, Millet GY, Wuyam B, and Verges S. Central and peripheral fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports* 22: 381-391, 2012.

33. di Prampero PE and Ferretti G. Factors limiting maximal oxygen consumption in humans. *Respir Physiol* 80: 113-127, 1990.

34. Dill DB, Myhre G, Phillips EE, Jr., and Brown DK. Work capacity in acute exposures to altitude. *J Appl Physiol* 21: 1168-1176, 1966.

35. Douglas CG, Halpane JS, Henderson Y, and Schneider EC. Physiological observations made on Pike's Peak, Colorada, with special reference to adaptation to low barometric pressures. *Philos T R Soc Lon B* 203: 185-318, 1913.

36. Ekblom B, Goldbarg AN, and Gullbring B. Response to exercise after blood loss and reinfusion. *J Appl Physiol* 33: 175-180, 1972.

37. Ekblom B, Huot R, Stein EM, and Thorstensson AT. Effect of changes in arterial oxygen content on circulation and physical performance. *J Appl Physiol* 39: 71-75, 1975.

38. Faoro V, Boldingh S, Moreels M, Martinez S, Lamotte M, Unger P, Brimioulle S, Huez S, and Naeije R. Bosentan decreases pulmonary vascular resistance and improves exercise capacity in acute hypoxia. *Chest* 135: 1215-1222, 2009.

39. Faoro V, Lamotte M, Deboeck G, Pavelescu A, Huez S, Guenard H, Martinot JB, and Naeije R. Effects of sildenafil on exercise capacity in hypoxic normal subjects. *High Alt Med Biol* 8: 155-163, 2007.

40. Fischler M, Maggiorini M, Dorschner L, Debrunner J, Bernheim A, Kiencke S, Mairbaurl H, Bloch KE, Naeije R, and Brunner-La Rocca HP. Dexamethasone but not tadalafil improves exercise capacity in adults prone to high-altitude pulmonary edema. *Am J Respir Crit Care Med* 180: 346-352, 2009.

41. Friedmann-Bette B. Classical altitude training. *Scand J Med Sci Sports* 18 Suppl 1: 11-20, 2008.

42. Friedmann B, Bauer T, Menold E, and Bartsch P. Exercise with the intensity of the individual anaerobic threshold in acute hypoxia. *Med Sci Sports Exerc* 36: 1737-1742, 2004.

43. Frostell CG, Blomqvist H, Hedenstierna G, Lundberg J, and Zapol WM. Inhaled nitric oxide selectively reverses human hypoxic pulmonary vasoconstriction without causing systemic vasodilation. *Anesthesiology* 78: 427-435, 1993.

44. Fulco CS, Rock PB, and Cymerman A. Maximal and submaximal exercise performance at altitude. *Aviat Space Environ Med* 69: 793-801, 1998.

45. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725-1789, 2001.

46. Gandevia SC, Allen GM, and McKenzie DK. Central fatigue. Critical issues, quantification and practical implications. *Adv Exp Med Biol* 384: 281-294, 1995.

47. Garvican LA, Pottgiesser T, Martin DT, Schumacher YO, Barras M, and Gore CJ. The contribution of haemoglobin mass to increases in cycling performance induced by simulated LHTL. *Eur J Appl Physiol* 111: 1089-1101, 2011.

48. Ghofrani HA, Reichenberger F, Kohstall MG, Mrosek EH, Seeger T, Olschewski H, Seeger W, and Grimminger F. Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. *Ann Intern Med* 141: 169-177, 2004.

49. Gibson AS and Noakes TD. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *Brit J Sport Med* 38: 797-806, 2004.

50. Gore CJ, Hahn A, Rice A, Bourdon P, Lawrence S, Walsh C, Stanef T, Barnes P, Parisotto R, Martin D, and Pyne D. Altitude training at 2690m does not increase total haemoglobin mass or sea level VO2max in world champion track cyclists. *J Sci Med Sport* 1: 156-170, 1998.

51. Gore CJ, Hahn AG, Aughey RJ, Martin DT, Ashenden MJ, Clark SA, Garnham AP, Roberts AD, Slater GJ, and McKenna MJ. Live high:train low increases muscle buffer capacity and submaximal cycling efficiency. *Acta Physiol Scand* 173: 275-286, 2001.

52. Gore CJ and Hopkins WG. Counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume. *J Appl Physiol* 99: 2055-2057; discussion 2057-2058, 2005.

53. Groves BM, Droma T, Sutton JR, McCullough RG, McCullough RE, Zhuang J, Rapmund G, Sun S, Janes C, and Moore LG. Minimal hypoxic pulmonary hypertension in normal Tibetans at 3,658 m. *J Appl Physiol* 74: 312-318, 1993.

54. Hammond MD, Gale GE, Kapitan KS, Ries A, and Wagner PD. Pulmonary gas exchange in humans during normobaric hypoxic exercise. *J Appl Physiol* 61: 1749-1757, 1986.

55. Henig NR and Pierson DJ. Mechanisms of hypoxemia. *Respir Care Clin N Am* 6: 501-521, 2000.

56. Hill AV, Long CHN, and Lupton H. Muscular exercise, lactic acid and the supply and utilisation of oxygen: parts I - III. *Proc R Soc Lond B Biol Sci* 96: 438–475, 1924.

57. Hill AV, Long CHN, and Lupton H. Muscular exercise, lactic acid and the supply and utilisation of oxygen: parts IV - VI. *Proc R Soc Lond B Biol Sci* 97: 84-138, 1924.

58. Hill AV, Long CHN, and Lupton H. Muscular exercise, lactic acid and the supply and utilisation of oxygen: parts VII - VIII. *Proc R Soc Lond B Biol Sci* 97: 155–176, 1924.

59. Hill AV and Lupton H. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Q J Med*: 135-171, 1923.

60. Hill JM, Pickar JG, Parrish MD, and Kaufman MP. Effects of hypoxia on the discharge of group III and IV muscle afferents in cats. *J Appl Physiol* 73: 2524-2529, 1992.

61. Hopkins WG and Hewson DJ. Variability of competitive performance of distance runners. *Med Sci Sports Exerc* 33: 1588-1592, 2001.

62. Horstman D, Weiskopf R, and Jackson RE. Work capacity during 3-wk sojourn at 4,300 m: effects of relative polycythemia. *J Appl Physiol* 49: 311-318, 1980.

63. Ide K, Eliasziw M, and Poulin MJ. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol* 95: 129-137, 2003.

64. Imray CH, Myers SD, Pattinson KT, Bradwell AR, Chan CW, Harris S, Collins P, and Wright AD. Effect of exercise on cerebral perfusion in humans at high altitude. *J Appl Physiol* 99: 699-706, 2005.

65. Joyner MJ and Coyle EF. Endurance exercise performance: the physiology of champions. *J Physiol* 586: 35-44, 2008.

66. Klausen K, Dill DB, and Horvath SM. Exercise at ambient and high oxygen pressure at high altitude and at sea level. *J Appl Physiol* 29: 456-463, 1970.

67. Kremenic IJ, Glace BW, Ben-Avi SS, Nicholas SJ, and McHugh MP. Central fatigue after cycling evaluated using peripheral magnetic stimulation. *Med Sci Sports Exerc* 41: 1461-1466, 2009.

68. Levine BD. .VO2max: what do we know, and what do we still need to know? *J Physiol* 586: 25-34, 2008.

69. Levine BD and Stray-Gundersen J. "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 83: 102-112, 1997.

70. Levine BD and Stray-Gundersen J. Point: positive effects of intermittent hypoxia (live high:train low) on exercise performance are mediated primarily by augmented red cell volume. *J Appl Physiol* 99: 2053-2055, 2005.

71. Levine BD and Stray-Gundersen J. A practical approach to altitude training: where to live and train for optimal performance enhancement. *Int J Sports Med* 13 Suppl 1: S209-212, 1992.

72. Linnarsson D, Karlsson J, Fagraeus L, and Saltin B. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* 36: 399-402, 1974.

73. Lundby C, Boushel R, Robach P, Moller K, Saltin B, and Calbet JA. During hypoxic exercise some vasoconstriction is needed to match O2 delivery with O2 demand at the microcirculatory level. *J Physiol* 586: 123-130, 2008.

74. Lundby C, Calbet JA, van Hall G, Saltin B, and Sander M. Pulmonary gas exchange at maximal exercise in Danish lowlanders during 8 wk of acclimatization to 4,100 m and in high-altitude Aymara natives. *Am J Physiol Regul Integr Comp Physiol* 287: R1202-1208, 2004.

75. Lundby C and Damsgaard R. Exercise performance in hypoxia after novel erythropoiesis stimulating protein treatment. *Scand J Med Sci Sports* 16: 35-40, 2006.

76. Lundby C, Millet GP, Calbet JA, Bartsch P, and Subudhi AW. Does 'altitude training' increase exercise performance in elite athletes? *Br J Sports Med*, 2012.

77. Lundby C, Moller P, Kanstrup IL, and Olsen NV. Heart rate response to hypoxic exercise: role of dopamine D2-receptors and effect of oxygen supplementation. *Clin Sci (Lond)* 101: 377-383, 2001.

78. Lundby C and Olsen NV. Effects of recombinant human erythropoietin in normal humans. *J Physiol* 589: 1265-1271, 2011.

79. Lundby C, Robach P, Boushel R, Thomsen JJ, Rasmussen P, Koskolou M, and Calbet JA. Does recombinant human Epo increase exercise capacity by means other than augmenting oxygen transport? *J Appl Physiol* 105: 581-587, 2008.

80. Lundby C, Sander M, van Hall G, Saltin B, and Calbet JA. Maximal exercise and muscle oxygen extraction in acclimatizing lowlanders and high altitude natives. *J Physiol* 573: 535-547, 2006.

81. Maffiuletti NA. Assessment of hip and knee muscle function in orthopaedic practice and research. *The Journal of bone and joint surgery American volume* 92: 220-229, 2010.

82. Maher JT, Jones LG, and Hartley LH. Effects of high-altitude exposure on submaximal endurance capacity of men. *J Appl Physiol* 37: 895-898, 1974.

83. Mellerowicz H, Meller W, Woweries J, Zerdick J, Ketusinh O, Kral B, and Heepe W. Verleichende Untersuchungen über die Wirkung von Höhentraining auf die Dauerleistung in Meereshöhe. *Sportarzt + Spormed* 21: 207-240, 1970.

84. Mosso A. *La Fatica*. Milano: Trevers, 1891.

85. Moudgil R, Michelakis ED, and Archer SL. Hypoxic pulmonary vasoconstriction. *J Appl Physiol* 98: 390-403, 2005.

86. Naeije R. Pulmonary circulation at high altitude. *Respiration* 64: 429-434, 1997.

87. Naeije R. Pulmonary circulation in hypoxia. *Int J Sports Med* 13 Suppl 1: S27-30, 1992.

88. Naeije R, Huez S, Lamotte M, Retailleau K, Neupane S, Abramowicz D, and Faoro V. Pulmonary artery pressure limits exercise capacity at high altitude. *Eur Respir J* 36: 1049-1055, 2010.

89. Naeije R and Rondelet B. Pathobiology of pulmonary arterial hypertension. *Bull Mem Acad R Med Belg* 159: 219-226, 2004.

90. Nootens M, Wolfkiel CJ, Chomka EV, and Rich S. Understanding right and left ventricular systolic function and interactions at rest and with exercise in primary pulmonary hypertension. *Am J Cardiol* 75: 374-377, 1995.

91. Powers SK, Lawler J, Dempsey JA, Dodd S, and Landry G. Effects of incomplete pulmonary gas exchange on VO2 max. *J Appl Physiol* 66: 2491-2495, 1989.

92. Pugh LG. Altitude and athletic performance. *Nature* 207: 1397-1398, 1965.

93. Pugh LG. Man at high altitude: studies carried out in the Himalaya. *Sci Basis Med Annu Rev*: 32-54, 1964.

94. Rasmussen P, Stie H, Nielsen B, and Nybo L. Enhanced cerebral CO2 reactivity during strenuous exercise in man. *Eur J Appl Physiol* 96: 299-304, 2006.

95. Read J and Fowler KT. Effect of Exercise on Zonal Distribution of Pulmonary Blood Flow. *J Appl Physiol* 19: 672-678, 1964.

96. Reynafarje C, Lozano R, and Valdivieso J. The polycythemia of high altitudes: iron metabolism and related aspects. *Blood* 14: 433-455, 1959.

97. Richalet JP, Gratadour P, Robach P, Pham I, Dechaux M, Joncquiert-Latarjet A, Mollard P, Brugniaux J, and Cornolo J. Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. *Am J Respir Crit Care Med* 171: 275-281, 2005.

98. Robach P, Calbet JA, Thomsen JJ, Boushel R, Mollard P, Rasmussen P, and Lundby C. The ergogenic effect of recombinant human erythropoietin on VO2max depends on the severity of arterial hypoxemia. *PLoS One* 3: e2996, 2008.

99. Robach P and Lundby C. Is live high-train low altitude training relevant for elite athletes with already high total hemoglobin mass? *Scand J Med Sci Sports* 22: 303-305, 2012.

100. Robach P, Schmitt L, Brugniaux JV, Nicolet G, Duvallet A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV, and Richalet JP. Living high-training low: effect on erythropoiesis and maximal aerobic performance in elite Nordic skiers. *Eur J Appl Physiol* 97: 695-705, 2006.

101. Robach P, Schmitt L, Brugniaux JV, Roels B, Millet G, Hellard P, Nicolet G, Duvallet A, Fouillot JP, Moutereau S, Lasne F, Pialoux V, Olsen NV, and Richalet JP. Living

high-training low: effect on erythropoiesis and aerobic performance in highly-trained swimmers. *Eur J Appl Physiol* 96: 423-433, 2006.

102. Robach P, Siebenmann C, Jacobs RA, Rasmussen P, Nordsborg N, Pesta D, Gnaiger E, Diaz V, Christ A, Fiedler J, Crivelli N, Secher NH, Pichon A, Maggiorini M, and Lundby C. The role of haemoglobin mass on VO2max following normobaric 'live high-train low' in endurance-trained athletes. *Br J Sports Med*, 2012.

103. Robertson EY, Saunders PU, Pyne DB, Aughey RJ, Anson JM, and Gore CJ. Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc* 42: 394-401, 2010.

104. Robertson RJ, Gilcher R, Metz KF, Caspersen CJ, Allison TG, Abbott RA, Skrinar GS, Krause JR, and Nixon PA. Effect of simulated altitude erythrocythemia in women on hemoglobin flow rate during exercise. *J Appl Physiol* 64: 1644-1649, 1988.

105. Robertson RJ, Gilcher R, Metz KF, Skrinar GS, Allison TG, Bahnson HT, Abbott RA, Becker R, and Falkel JE. Effect of induced erythrocythemia on hypoxia tolerance during physical exercise. *J Appl Physiol* 53: 490-495, 1982.

106. Roca J, Hogan MC, Story D, Bebout DE, Haab P, Gonzalez R, Ueno O, and Wagner PD. Evidence for tissue diffusion limitation of VO2max in normal humans. *J Appl Physiol* 67: 291-299, 1989.

107. Romer LM, Haverkamp HC, Lovering AT, Pegelow DF, and Dempsey JA. Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans. *Am J Physiol Regul Integr Comp Physiol* 290: R365-375, 2006.

108. Sahlin K. Muscle fatigue and lactic acid accumulation. *Acta Physiol Scand Suppl* 556: 83-91, 1986.

109. Saltin B and Calbet JA. Point: in health and in a normoxic environment, VO2 max is limited primarily by cardiac output and locomotor muscle blood flow. *J Appl Physiol* 100: 744-745, 2006.

110. Saltin B and Strange S. Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. *Med Sci Sports Exerc* 24: 30-37, 1992.

111. Saunders PU, Pyne DB, and Gore CJ. Endurance training at altitude. *High Alt Med Biol* 10: 135-148, 2009.

112. Saunders PU, Telford RD, Pyne DB, Cunningham RB, Gore CJ, Hahn AG, and Hawley JA. Improved running economy in elite runners after 20 days of simulated moderate-altitude exposure. *J Appl Physiol* 96: 931-937, 2004.

113. Schuler B, Thomsen JJ, Gassmann M, and Lundby C. Timing the arrival at 2340 m altitude for aerobic performance. *Scand J Med Sci Sports* 17: 588-594, 2007.

114. Siebenmann C, Bloch KE, Lundby C, Nussbamer-Ochsner Y, Schoeb M, and Maggiorini M. Dexamethasone improves maximal exercise capacity of individuals susceptible to high altitude pulmonary edema at 4559 m. *High Alt Med Biol* 12: 169-177, 2011.

115. Siebenmann C, Bloch KE, Lundby C, Nussbamer-Ochsner Y, Schoeb M, and Maggiorini M. Maximal exercise capacity in individuals susceptible to high altitude pulmonary edema at 4559 m.

116. Siebenmann C, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M, and Lundby C. "Live high-train low" using normobaric

hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol* 112: 106-117, 2012.

117. Siebenmann C, Sørensen H, Jacobs RA, Haider T, Rasmussen P, and Lundby C. Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O2 uptake. *Respir Physiol Neurobiol* (In press), 2012.

118. Singh MV, Rawal SB, and Tyagi AK. Body fluid status on induction, reinduction and prolonged stay at high altitude of human volunteers. *Int J Biometeorol* 34: 93-97, 1990.

119. Staub NC. Site of hypoxic pulmonary vasoconstriction. *Chest* 88: 240S-245S, 1985.

120. Stickland MK, Welsh RC, Haykowsky MJ, Petersen SR, Anderson WD, Taylor DA, Bouffard M, and Jones RL. Effect of acute increases in pulmonary vascular pressures on exercise pulmonary gas exchange. *J Appl Physiol* 100: 1910-1917, 2006.

121. Subudhi AW, Lorenz MC, Fulco CS, and Roach RC. Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. *Am J Physiol Heart Circ Physiol* 294: H164-171, 2008.

122. Subudhi AW, Miramon BR, Granger ME, and Roach RC. Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. *J Appl Physiol* 106: 1153-1158, 2009.

123. Subudhi AW, Olin JT, Dimmen AC, Polaner DM, Kayser B, and Roach RC. Does cerebral oxygen delivery limit incremental exercise performance? *J Appl Physiol* 111: 1727-1734, 2011.

124. Sun XG, Hansen JE, Oudiz RJ, and Wasserman K. Exercise pathophysiology in patients with primary pulmonary hypertension. *Circulation* 104: 429-435, 2001.

125. Sutton JR, Reeves JT, Wagner PD, Groves BM, Cymerman A, Malconian MK, Rock PB, Young PM, Walter SD, and Houston CS. Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *J Appl Physiol* 64: 1309-1321, 1988.

126. Svedenhag J, Piehl-Aulin K, Skog C, and Saltin B. Increased left ventricular muscle mass after long-term altitude training in athletes. *Acta Physiol Scand* 161: 63-70, 1997.

127. Sylvester JT, Shimoda LA, Aaronson PI, and Ward JP. Hypoxic pulmonary vasoconstriction. *Physiol Rev* 92: 367-520, 2012.

128. Ting H, Sun XG, Chuang ML, Lewis DA, Hansen JE, and Wasserman K. A noninvasive assessment of pulmonary perfusion abnormality in patients with primary pulmonary hypertension. *Chest* 119: 824-832, 2001.

129. Tiollier E, Schmitt L, Burnat P, Fouillot JP, Robach P, Filaire E, Guezennec C, and Richalet JP. Living high-training low altitude training: effects on mucosal immunity. *Eur J Appl Physiol* 94: 298-304, 2005.

130. Tolle JJ, Waxman AB, Van Horn TL, Pappagianopoulos PP, and Systrom DM. Exercise-induced pulmonary arterial hypertension. *Circulation* 118: 2183-2189, 2008.

131. Torre-Bueno JR, Wagner PD, Saltzman HA, Gale GE, and Moon RE. Diffusion limitation in normal humans during exercise at sea level and simulated altitude. *J Appl Physiol* 58: 989-995, 1985.

132. Wagner PD. Counterpoint: in health and in normoxic environment VO2max is limited primarily by cardiac output and locomotor muscle blood flow. *J Appl Physiol* 100: 745-747; discussion 747-748, 2006.

133. Wagner PD. Modeling O2 transport as an integrated system limiting VO2MAX. *Comput Methods Programs Biomed* 101: 109-114, 2011.

134. Wagner PD. The physiological basis of reduced VO2max in Operation Everest II. *High Alt Med Biol* 11: 209-215, 2010.

135. Wagner PD. Reduced maximal cardiac output at altitude--mechanisms and significance. *Respir Physiol* 120: 1-11, 2000.

136. Walker S, Peltonen J, Ahtiainen JP, Avela J, and Hakkinen K. Neuromuscular fatigue induced by an isotonic heavy-resistance loading protocol in knee extensors. *J Sports Sci* 27: 1271-1279, 2009.

137. Wax D, Garofano R, and Barst RJ. Effects of long-term infusion of prostacyclin on exercise performance in patients with primary pulmonary hypertension. *Chest* 116: 914-920, 1999.

138. Wensel R, Opitz CF, Ewert R, Bruch L, and Kleber FX. Effects of iloprost inhalation on exercise capacity and ventilatory efficiency in patients with primary pulmonary hypertension. *Circulation* 101: 2388-2392, 2000.

139. West JB. Respiratory and circulatory control at high altitudes. *J Exp Biol* 100: 147-157, 1982.

140. West JB, Boyer SJ, Graber DJ, Hackett PH, Maret KH, Milledge JS, Peters RM, Jr., Pizzo CJ, Samaja M, Sarnquist FH, and et al. Maximal exercise at extreme altitudes on Mount Everest. *J Appl Physiol* 55: 688-698, 1983.

141. Wilber RL and Pitsiladis YP. Kenyan and Ethiopian distance runners: what makes them so good? *Int J Sports Physiol Perform* 7: 92-102, 2012.

142. Wolfel EE, Groves BM, Brooks GA, Butterfield GE, Mazzeo RS, Moore LG, Sutton JR, Bender PR, Dahms TE, McCullough RE, and et al. Oxygen transport during steady-state submaximal exercise in chronic hypoxia. *J Appl Physiol* 70: 1129-1136, 1991.

143. Zuntz N, Loewy A, Müller F, and Caspari W. *Höhenklima und bergwanderungen in ihrer wirkung auf den menschen Ergebnisse experimenteller Forschungen im Hochgebirge und Laboratorium*. Berlin: Deutsches Verlagshaus Bong, 1906.

5. Curriculum Vitae

Name: Siebenmann

Forenames: Christoph Andreas

Date of Birth: 23. 04. 1982

Place of Birth: Zurich, Switerland

Education:

1989 - 1995	Elementary school, Zumikon, Switzerland
1995 - 2002	Gymnasium Hohe Promenade, Zurich, Switzerland: Matura-Typus B
2002 - 2007	Swiss Federal Institute of Technology, Zurich, Switzerland: Bachelor course in Human Movement Sciences
2007 - 2008	Swiss Federal Institute of Technology, Zurich, Switzerland: Master course in Human Movement Sciences with focus on exercise physiology
2009 - 2010	University hospital of Zurich, Switzerland: PhD-study
2010 - 2012	Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland: PhD-study

Publications:

Original publications:

1. Jacobs RA, Rasmussen P, **Siebenmann C**, Diaz V, Gassmann M, Pesta D, Gnaiger E, Nordsborg NB, Robach P, and Lundby C. Determinants of time trial performance and maximal incremental exercise in highly trained endurance athletes. *J Appl Physiol* 111: 1422-1430, 2011.

2. Nordsborg NB, **Siebenmann C**, Jacobs RA, Rasmussen P, Diaz V, Robach P, and Lundby C. Four weeks of normobaric "live high-train low" do not alter muscular or systemic capacity for maintaining pH and K+ homeostasis during intense exercise. *J Appl Physiol* 112: 2027-2036, 2012.

3. Nussbaumer-Ochsner Y, Schuepfer N, **Siebenmann C**, Maggiorini M, and Bloch KE. High altitude sleep disturbances monitored by actigraphy and polysomnography. *High Alt Med Biol* 12: 229-236, 2011.

4. Nussbaumer-Ochsner Y, Ursprung J, **Siebenmann C**, Maggiorini M, and Bloch KE. Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. *Sleep* 35: 419-423, 2012.

5. Robach P, **Siebenmann C**, Jacobs RA, Rasmussen P, Nordsborg N, Pesta D, Gnaiger E, Diaz V, Christ A, Fiedler J, Crivelli N, Secher NH, Pichon A, Maggiorini M, and

Lundby C. The role of haemoglobin mass on VO2max following normobaric 'live high-train low' in endurance-trained athletes. *British journal of sports medicine* 2012 (in press).

6. **Siebenmann C**, Bloch KE, Lundby C, Nussbamer-Ochsner Y, Schoeb M, and Maggiorini M. Dexamethasone improves maximal exercise capacity of individuals susceptible to high altitude pulmonary edema at 4559 m. *High Alt Med Biol* 12: 169-177, 2011.

7. **Siebenmann C**, Robach P, Jacobs RA, Rasmussen P, Nordsborg N, Diaz V, Christ A, Olsen NV, Maggiorini M, and Lundby C. "Live high-train low" using normobaric hypoxia: a double-blinded, placebo-controlled study. *J Appl Physiol* 112: 106-117, 2012.

8. **Siebenmann C,** Sørensen H, Jacobs RA, Haider T, Rasmussen P, and Lundby C. Hypocapnia during hypoxic exercise and its impact on cerebral oxygenation, ventilation and maximal whole body O2 uptake. *Respir Physiol Neurobiol*, 2012 (in press).

9. Sorensen H, Secher NH, **Siebenmann C,** Nielsen HB, Kohl-Bareis M, Lundby C, and Rasmussen P. Cutaneous Vasoconstriction Affects Near-infrared Spectroscopy Determined Cerebral Oxygen Saturation during Administration of Norepinephrine. *Anesthesiology* 117: 263-270, 2012.

Reviews and replies:

6.a. **Siebenmann C**, and Maggiorini M. Dexamethasone Improves Maximal Exercise Capacity of Individual's Susceptible to High Altitude Pulmonary Edema at 4559 m Reply. *High Altitude Medicine & Biology* 12: 413-413, 2011.

7.a. Lundby C, Pichon A, and **Siebenmann C**. Ineffective normobaric LHTL: room confinement or inappropriate training intensity? Reply. *Journal of Applied Physiology* 112: 528-528, 2012.

7.b. Lundby C, **Siebenmann C**, and Robach P. Hemoglobin mass response to simulated hypoxia "blinded" by noisy measurement? Reply. *Journal of Applied Physiology* 112: 1799-1799, 2012.